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" THE CORRELATION OF TEAR FUNCTION TESTS AND CONJUNCTIVAL IMPRESSION CYTOLOGY IN DRY EYES AMONG PATIENTS ATTENDING SANTOSH MEDICAL HOSPITAL , GHAZIABAD "

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ABBREVIATIONS

- AC- Abnormal Cytology
- CIC-Conjunctival Impression Cytology.
- DED-Dry Eye Diseases.
- DES-Dry eye severity.
- DEWS- Dry Eyes Work Shop.
- GCD-Goblet cell density.
- H&E-Haematoxylin and eosin.
- IC- Impression Cytology
- IBD- Inflammatory bowel disease.
- KCS-Keratoconjunctivitis sicca
- NC- Normal Cytology.
- OSDI- Ocular Surface Disease Index.
- PAS-Periodic Acid Schiff.
- RBS-Rose Bengal Stain.
- RGP-Rigid gas permeable.
- ST-Schirmers Test.
- SPK-Superficial Punctate Keratitis
- TBUT-Tear Film Break Up Time
- VBS-Van Bijsterveld Score

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INTRODUCTION

The International Dry Eye Workshop defines dry eye as a multifactorial disease of the tears and ocular surface that causes symptoms such as discomfort, visual disruption, and tear film instability, as well as potential ocular surface damage. Along with it, the tear film's osmolarity increases, and the ocular surface becomes inflamed.[1]

The presence of dry eye disease and its effects on patient's quality of life makes it a significant healthcare issue.Dry eye prevalence rises with advancing age. According to estimates, dry eye syndrome will affect approximately 75% of adults over 65 years.[2] Dry eye prevalence ranged from 18.4 to 40.8% in India.[3,4,5]

Despite tremendous advancements, our understanding of dry eye aetiology is continually evolving, each test only looks at a small portion of a process, the findings from several tests together can deliver a more comprehensive picture of this dynamic process. There is no specific test which can analyse the ocular tear film thoroughly.[6]

The absence of a widely accepted gold standard test has made it more challenging for ophthalmologists to diagnose and treat dry eye clinically. As a result, symptom-based assessment has increasingly become a crucial diagnostic element.[7]

Any disruption to the tear film will lead it to become dysfunctional, leading to characteristic symptoms and signs of dry eye syndrome.Ocular damage in dry eyes is reversible, if diagnosed and treated early or else may lead to complications like corneal abrasion, recurrent inflammations, keratitis, corneal perforation, corneal scarring, corneal thinning, irreversible visual impairment.

Slit lamp examination, Schirmer's test, tear film break up time, and Rose Bengal staining are all used to establish a clinical diagnosis of dry eye. Conjunctival biopsy is the traditional method for examining the histopathological abnormalities.

Since it is invasive, this technique is not regularly performed. Conjunctival impression cytology, a more contemporary test, has been created to acquire conjunctival impressions and examine cellular morphology. It uses a filter paper approach with millipore cellulose acetate paper.

The Schirmer's test is the most commonly used method of measuring tear production. Due to reflex tearing, it cannot be repeated and ignores the evaporative features of dry eye. Although Schirmer's test performed without anaesthesia is a viable alternative for extremely dry eyes, it lacks sensitivity.[8] On the other hand, tear film stability can be evaluated using the tear film break up time (TBUT). Considering that it can be somewhat repeated, this test appears to be more accurate than Schirmer's.[9,10]

The level of ocular surface damage is measured by staining with essential dyes like Rose Bengal, but this method might miss milder cases. In dry eye states, it can also be used in conjunction with other tests like the TBUT.[11] Patients do not tolerate conjunctival excision biopsy, and in clinical practise, not all facilities may be equipped to execute the procedure.[12]

A qualitative approach to assess the alterations in conjunctival morphology and goblet cell density in dry eyes is conjunctival impression cytology.[13]

In 1954, Larmande and Timsit originally characterised it as a diagnostic tool for squamous neoplasia of the ocular surface.[14] In 1977, Egbert et al. published a report on the application of impression cytology for assessing goblet cell densities and ocular surface diseases.[15]

Tsing changed the procedure for collecting the specimens and stained them using PAS and Papanicolaou dyes together. Impression cytology is used for a variety of purposes, including the aetiological diagnosis of various ocular surface disorders such as dry eye, recording consecutive alterations in the conjunctival and corneal surface over time, assessing therapeutic effects and staging conjunctival squamous metaplasia, and as an investigative tool for analysing ocular surface disease with immunostaining and DNA analysis.[16]

Maskin and Bodé established a method for examining conjunctival epithelial cells obtained from Reward reward minutes impression cytology using electron microscopy.[17]

The corneal and conjunctival epithelium of the ocular surface is composed of 5-7 layers of polygonal cells and is flat, multi-stratified, and non-keratinized. The conjunctiva also contains a large number of goblet cells that help the lacrimal film to develop.

This epithelium goes through reversible adaptive changes in conditions like dry eye, which may or may not be accompanied by inflammation. One of these alterations is a pathological shift to a conjunctival and corneal surface that is keratinized and non-secretory and is marked by the loss of goblet cells.[18,19,20]

In conjunctival impression cytology, the ocular surface's morphology is preserved while the outer layers of the conjunctival epithelium are collected for morphological analysis. It is repeatable, minimally invasive, offers the opportunity to track the progression of morphological changes, and provides accurate information on the area sampled with little discomfort to the patient.[21,22,23]

This technique can be used to analyse conjunctival goblet cells, which are decreased in people with severe dry eye, as well as to identify early abnormalities in the process of squamous metaplasia before they become clinically obvious and permanent.[24]

It is a commonly used approach for studying the ocular surface in diseases such as viral conjunctivitis, keratoconjunctivitis sicca,[25,26] vitamin A insufficiency,[27] chronic renal failure, psoriasis, ocular surface tumors.[28] However, its greatest benefit has been in the treatment of dry eye condition, when goblet cells are depleted.

For determining the severity of dry eye and making a diagnosis, it is just as reliable as a biopsy. It aids in determining the level of dry eye severity. The conjunctival surface can be examined using this very sensitive technique to identify pathological alterations and support a clinical diagnosis.

However, it is still not the foremost investigation in dry eye syndrome. Currently, these tests are frequently used in conjunction to diagnose and grade the severity of dry eye.

Conjunctival impression cytology applications in dry eye syndromes include:

1-Dry eye diagnosis
2. To determine the severity level.
3. To understand how dry eyes affect the ocular surface
4-To evaluate how a treatment will affect the prognosis and the course of the disease.
5-To find another ocular surface pathology that is related.

Considering the advantages of this minimally invasive technique over other methods , the goal of the current study is to determine the correlation between cytology grading and clinical tests for dry eyes caused by various etiologies and in healthy individuals, to assess the degree to which these tests correlate with dry eye symptoms in order to determine whether the cytological test can be alternative to conjunctival biopsy in the diagnosis and prognosis of dry eye syndromes.

AIM AND OBJECTIVES

AIM-

The study entitled "The correlation of tear function tests and conjunctival impression cytology in dry eyes among patients attending Santosh Medical Hospital, Ghaziabad" present with the following objectives:

OBJECTIVES-

1-To determine a correlation between clinical and cytological tests used to assess the severity of dry eye.

2-To assess the outcomes of conjunctival impression cytology in patients with dry eye and in normal individuals.

REVIEW OF LITERATURE

Keratoconjunctivitis sicca (KCS), often known as dry eye disease (DED), is among the most prevalent ophthalmologic diseases. According to epidemiologic studies, the prevalence of diagnosed DED was found to be 0.4-0.5% overall,[29] and was highest among women and primarily in the elderly age group.[29,30] The majority of those affected have less severe symptoms, and they likely present with more of an episodic manifestation of the disease that is notable only when adverse contributing conditions, like low humidity or contact lens wear.. DED will therefore have a major economic impact on both direct and indirect expenditures across the country, making it a significant public health issue.[29,30]

In 2022, Belviranli S. carried out a study to compare the tear film characteristics and conjunctival impression cytology (CIC) findings of patients with multiple sclerosis (MS) to controls. The MS group received a mean CIC grade that was higher than the control group's (1.48 ± 0.71 and 0.39 ± 0.56, respectively; $p < 0.001$). Of the 14 participants in the MS group, CIC was in grades 2-3 for 42.4% of them. The only participant (3.3%) in the control group had a CIC that was in grade 2, and none of the participants had a CIC that was in grade 3. The TBUT (8.12 ± 3.16, 13.06 ± 4.23 s), and Schirmer 1 test results (8.45 ± 5.75, 17.36 ± 10.89) mm, respectively, in MS and control groups, respectively, were lower, and the OSDI score (in MS and control groups, respectively, ($p < 0.001$).Objective signs of dry eyes, subjective symptoms associated with dry eyes, and CIC abnormalities, such as severe conjunctival squamous metaplasia and goblet cell loss, are more prevalent in MS patients.[31]

In 2021, Valencia Castillo SL did a study with the goal of analysing alterations in conjunctival impression cytology with transfer and both lachrymal stability and flow tests in individuals with dry eye illness following therapy with autologous serum eye drops. The study included 24 patients (23 women, mean age 53.8 ± 12.6 years). Six (25.0%) and ten (41.7%) of the patients had severe dry eye disease. There was rheumatoid arthritis in five cases. Conjunctival goblet cell size, density, nucleus size, and nucleus/cytoplasm ratio all significantly improved after therapy (202.3 ± 107.5 vs 210.1 ± 100.9 cells/mm2, $p<0.01$). The degree of metaplasia improved in seven out of ten individuals with grade 3 or 4 metaplasia. Schirmer's test and tear break-up time both saw considerable improvements.In this patient subgroup, there was a notable improvement in tear break-up time and Schirmer's test results. Conjunctival goblet cell

proliferation was linked to the quantity of goblet cells in the multivariate analysis.[32]

In 2020, Usuba FS conjunctival goblet cells were used as an early ocular biomarker in a study conducted by on dry eyes in rheumatoid arthritis patients using TNF-inhibitors. Patients with rheumatoid arthritis (RA) frequently experience dry eye disease (DED). Patients with rheumatoid arthritis receiving TNF-inhibitors (TNFi) therapy can use conjunctival goblet cell count as a clinical biomarker to identify their condition and gauge how well they are responding to treatment. The purpose of this study was to look into the ocular surface parameters as well as the long-term effects of TNFi therapy on rheumatoid arthritis patients' ocular surface features and goblet cell count. As a biomarker of the disease process and treatment response in this population, TNFi rapidly increases tear production and restores the goblet cells.[33]

In 2020, Turan M studied the ocular surface by impression cytology in endometriosis patients. The average Schirmer I test findings in group 1 were 8.40 ± 2.74 mm, while endometriosis patients had significantly lower values (P <0.001). The average TBUT test findings in group 1 were 9.04 ± 3.61s, while endometriosis patients had considerably lower scores (P <0.001). The average OSDI scores in group 1 were 24.04 ± 9.29 , and endometriosis patients had considerably higher scores (P 0.001). In group 1, the average CIC results were 1.76 ± 0.88. Patients with endometriosis had considerably higher average CIC levels (P <0.001). Patients with endometriosis may exhibit ocular surface alterations, including squamous metaplasia, in their conjunctiva.[34]

In 2020, Dikci S conducted a study to assess the functioning of the tear film and conjunctival surface cells in people who were vitamin D deficient. In the study group, the average serum vitamin D levels were10.5 ± 5.0 µl, while in the control group, they were 33.9 ± 11.7 (p <0.001). In the study and control groups, the median TBUT scores were 11 and 17 seconds, and the median Schirmer II values were 7.5 and 12 millimetres, respectively (p = 0.003, p = 0.049). 69.4% of the patients in the study group had impression cytology classified as grade 2 or 3 according to the Nelson staging system, compared to 18.5% of the participants in the control group (p< 0.001).The findings of the study suggested that a vitamin D deficit may result in dry eye, which damages the ocular surface's goblet cells and causes conjunctival squamous metaplasia.[35]

In 2019, Thia ZZ updated research on the function of impression cytology in ocular surface diseases was done in Ocular surface disease (OSD molecular)pathogenesis is poorly understood, and its current management is entirely inadequate. Impression cytology (IC), a relatively benign method, has proven to be helpful in the treatment of OSD. The technologies used in OSD research studies using IC have significantly advanced in recent years, and standardised IC is even being used in dry eye clinical trials. This study serves as an update on the development in this field since the previous significant evaluation of IC by describing the advancements of IC over the past ten years. The improvements in IC have allowed for a more thorough assessment of the ocular surface, allowing for greater knowledge and management of OSD.[36]

In 2019, Zakharova NM performed a study on the use of impression cytology in the diagnosis of dry eye condition. Analysis of the cellular makeup of the conjunctival prints on the eyeball and assessment of the usefulness of the cytomorphological research method in the detection of dry eye syndrome (DES). 72 patients underwent cytological evaluation using impression cytology: 60 had DES and 12 had no eye illness (control group). Squamous epithelium (hyperkeratocytes), groups and clusters of flattened epithelial cells, and a limited number (up to five in the preparation) of goblet cells make up the majority of the cytological sample in individuals with DES. The accuracy of DES diagnosis is improved by the use of impression cytology.[37]

In 2018, Doğan M conducted a study to assess the ocular surface alterations and tear-film functioning in patients with inflammatory bowel disease (IBD). The Schirmer I test revealed that the rates of dry eyes in the IBD and control groups were 56.1% and 10.3%, respectively ($P< 0.001$). Through TBUT, the prevalence of dry eye was 52.6% in the IBD group and 20.8% in the control group, respectively ($P=0.004$). The IBD group had substantially higher Ocular Surface Disease Index scores (15.25 ± 7.31) than the control group (11.75 ± 7.33; $P=0.039$). The Oxford method revealed that 6.9% of the control group and 54.4% of the IBD group had mild-to-moderate staining, respectively ($P <0.001$). 69% of the IBD group had stage 2 or stage 3 impression cytology, according to the Nelson Staging System, while none of the controls had stage 2 or stage 3 impression cytology ($P <0.001$).According to the Nelson Staging System, 69% of the IBD group had stage 2 or stage 3 impression cytology, while the control group had neither ($P <0.001$).[38]

In 2018, Guannan H conducted study to evaluate the clinical traits and alterations in ocular surface cytology of dry eye in patients with systemic autoimmune illness. The two groups each had 60 (or 50%) subjects. In the control group, the prevalence of dry eye was 17 (28.3%), but it was 31 (51.7%) in the patient group ($p < 0.01$). Conjunctival impression sampling revealed that among dry eye patients, the severity level of the cells was substantially higher than in controls ($p < 0.01$). In both groups, patients with dry eye had a larger percentage of conjunctival epithelial cells undergoing apoptosis than patients without dry eye, and among patients with dry eye, patients had a higher rate of apoptosis than controls.Patients with dry eye associated with systemic autoimmune illness had more severe ocular surface cell injury than subjects with dry eye associated with healthy controls.[39]

In 2018, Oltulu P conducted a study to compare the symptoms of dry eye in patients with ulcerative colitis (UC) and healthy individuals. When compared to healthy persons, patients with UC had reduced tear volumes ($P < 0.05$). Between groups 1 and 2, there was a statistically significant variation in the distribution of CIC grades ($P < 0.05$). Schirmer-I measures and tear break-up time were both considerably reduced in group 1 compared to group 2 ($P < 0.05$). The findings of this study suggest that UC is linked to observable abnormalities in conjunctival cytology and tear function.[40]

In 2017, Weber SP carried a study to examine how dry eye disease patients wearing scleral contacts fared on conjunctival impression cytology evaluation. ScCLs were implanted in 41 eyes from 25 patients to treat DED. Stevens-Johnson syndrome (22 eyes), Sjogren's syndrome (11 eyes), graft-versus-host disease (2 eyes), dry eye following keratomileusis (2 eyes), and undifferentiated ocular surface disease (2 eyes) were the underlying illnesses (4 eyes). With the exception of Sjogren syndrome, where there was an unexpected rise in HLA expression, the ScCLs had no effect on the criteria used to assess inflammatory processes, which were determined by conjunctival impression cytology and HLA-DR expression.[41]

In 2016, Mocanu CL did a survey on Impression cytology in sicca syndrome to compare clinical and cytological findings related to dry eye syndromes and found a substantial correlation between the clinical grade of disease severity and the degree of squamous metaplasia involving goblet cell loss. He came to the conclusion that impression cytology is simple to use and quickly identifies changes in the tear film with excellent specificity and sensitivity.[42]

In 2015, Rahul Bhargava studied the differences between CIC and standard tear function testing for contact lens wearers in 2015. Nelson grade substantially linked with complaints of dry eyes in patients in contrast to controls (r2 = 0.010) (r2 = 0.765). However, he discovered a modest correlation among controls (r2 = 0.130 and 0.054) and a substantial correlation for dry eye symptoms, Schirmer test, and TBUT among cases (r2 = 0.557 and 0.530, respectively). The TBUT's sensitivity was discovered to be 86.4%, specificity 82.4%, and a positive likelihood ratio (LR) of 4.50 (95% confidence interval (CI) 3.46-5.85), and the low LR value was 0.09. The Schirmer test was shown to have a 48.2% sensitivity, an 88% specificity, a negative LR 2.12 (95% CI 1.48-2.96), and LR 0.83 is negative. When compared to the Schirmer test and TBUT, he found that CIC will more closely correlate with the symptoms of dry eyes.[43]

In 2014, Kumar P conducted a study to determine the strength of the connection involving routine tear function tests and conjunctival impression cytology, as well as the sensitivity and specificity of these tests when CIC is considered the gold standard. The average Schirmer's value for patients was 11.66 ± 5.90 and for controls was 17.17 ± 2.97 ($p < 0.001$). The mean TBUT for participants was 8.88 ± 3.54 while for controls it was 13.53 ± 2.12 ($p < 0.001$). The mean goblet cell density (GCD) in the patients was 490 ± 213 while it was 1,462 ± 6611 in the controls ($p < 0.001$). 32.8% of controls and 46.7% of dry eye cases both had abnormal CIC. Schirmer's correlation coefficient (L) for participants and controls was 0.2 and 0.24, whilst TBUT values for cases and controls were 0.26 and 0.38, RBS values were 0.5 and 0.5, and GCD values were 0.8 and 0.6. He came to the conclusion that Rose Bengal stain and TBUT are more accurate predictors of dry eye with sensitivity than Schirmer's. When CIC considered the gold standard, Schirmer's > TBUT > Rose Bengal stain and specificity were Schirmer's > TBUT > Rose Bengal stain in decreasing order.[44]

In 2014, Zuazo F used conjunctival impression cytology (CIC) to describe goblet cell density and Nelson grading in distinct locations of the ocular surface in subjects with normal and damaged Ocular Surface Disease Index (OSDI) scores. Individuals with a highly impaired OSDI score had a higher Nelson grading (1.41±0.14) compared to patients without severe impairment(0.86±0.09) ($P<.01$). When compared to healthy participants (497.31±50.07 cells per sample), goblet cell density was considerably lower in individuals with a significantly impaired OSDI score (310.24±56.24 cells per sample; P.001). Both in patients with mild ($P<.01$) and moderate ($P<.001$) OSDI scores, goblet cell density on the non-photoexposed

conjunctiva was considerably higher than on the photoexposed bulbar conjunctiva. Less goblet cells and a higher Nelson score can be found in patients with severe OSDI impairment.[45]

In 2013, Kumar S. conjunctival impression cytology was used in a study by on computer users. The control group's conjunctival impression cytology (CIC) findings ranged from stage 0 to stage 1, while the computer user group's CIC results ranged from stage II to stage IV. The majority (> 90%) of computer users displayed stage III and stage IV alterations. It demonstrated that people who worked at computers for longer periods of time each day than they did each day saw greater CIC alterations.[46]

The 2011 Meibomian Gland Dysfunction International Workshop covered a wider range of topics regarding the pathophysiology and treatment of MGD.[47]

In 2011, Shresta did a study to compare the findings of conjunctival impression cytology comparing dry eye patients and healthy individuals. Nelsons Classification studied cytological classification, while Murube Del Clinical Classification studied clinical classification. He discovered that dry eye illness was more common in women. 49.2% of the 114 dry eye cases exhibited decreased or nonexistent goblet cell density. In 72 healthy people, 73.7% of eyes displayed normal goblet cell density, while 26.3% of eyes displayed fewer or nonexistent goblet cells ($p < 0.001$) In cases compared to controls, the tear break-up time (TBUT) test was substantially more likely to be fewer than 10 seconds (OR = 19.36, 95% CI = 7.56 - 52.52). The goblet cell density was also probably much lower in instances of dry eye syndrome (OR= 2.25, 95% CI = 1.26 - 4.02, $p = 0.003$). He came to this conclusion because dry eyes had much less goblet cells than healthy eyes.[48]

In 2009, Lopin E conducted research on Impression cytology: current advancements and applications in dry eye illness . Cells can be collected non-invasively from the ocular surface using impression cytology (IC). Using cellulose acetate filters or Biopore membranes to remove the epithelium's outermost layers allows for the analysis of the cells using a variety of techniques, depending on the investigation's or pathology's goals. The patient experiences almost no pain when IC procedures are used, they are simple to learn, and they may be done in an outpatient setting. Ocular surface problems such keratoconjunctivitis sicca, ocular surface squamous neoplasia, and ocular surface infections are all easier to diagnose with IC. In the past ten years, IC has been used more and more to help with ocular surface disease diagnosis,

enhance our knowledge of the pathogenesis of ocular surface disease and offer biomarkers for use as clinical trial outcome measurements. One area where IC has made a big contribution to progress is dry eye disease.[49]

In 2007, the International Dry Eye Workshop (DEWS) updated the concept of DES and provided more empirical understanding and knowledge. [50]

During 2004-2006, an International Task Force - DELPHI consensus panel on Dysfunctional Tear Syndrome was held, and for the first time, a suggested level approach to treating dry eye illnesses was developed. [51]

In 2003, Murube J conducted a study to determine the relationship between squamous metaplasia and clinical severity, on impression cytology on the conjunctiva and cornea in dry eye patients. According to his research, nonsecretory epithelial cells of the conjunctiva and cornea, as well as goblet cell densities from the conjunctiva, showed significant differences between clinically normal eyes and those with the five grades of clinical severity of dry eye, with varying degrees of squamous metaplasia. These differences were primarily in cell sizes, nuclear alterations, and the N:C ratio. He comes to the conclusion that the clinical severity of dry eye coincides with the cytological changes and that morphological tests on ocular surface cells show a substantial difference between clinically normal eyes and those with the five degrees of dry eye severity.[52]

In 2002, Yaylali V did a study comparing ocular results in acne rosaceae with tear function tests and impressions. Itching (80%), redness (68%), burning (64%), and photophobia (60%) were the most prevalent symptoms. Meibomitis (92%), telangiectasis (88%), blepharitis (84%), superficial punctate keratopathy (72%), and hyperemia (60%), were the most prevalent clinical findings. The average tear BUT measured 9.6+/- 3.1 (SD) seconds (range 5-17 seconds). The mean Schirmer test was 11.4+/-1.6 mm (SD) in length (range 8-15 mm). 1.8+/-0.1 was the average rose Bengal staining score. The rosacea group had lower mean tear BUT, Schirmer test, and rose Bengal staining grades than the control group ($p=0.003$, $p=0.04$, and $p=0.038$, respectively). 18 individuals (36%) had pathologic staining, with a staining score of 4 being the highest. Each eye's nasal and temporal conjunctival impression cytology stages did not significantly differ from one another, and there was no correlation between these phases and

rose Bengal scores ($p>0.05$). He came to the conclusion that Impression cytology can be utilised effectively in the early diagnosis of dry eye in conjunction to tear function tests.[53]

In 2002, Simon carried out a study on contact lens wearers' conjunctival impression cytology. With time spent wearing contacts, cytological alterations became more severe ($P = 0.00001$). 60% of symptomatic eyes using soft contact lenses and RGP lenses displayed abnormal CIC changes after six months. None of the asymptomatic eyes wearing RGP contact lenses had abnormal CIC changes, while 33.4% of the asymptomatic eyes wearing soft contacts did ($P = 0.033$). Within three to six months of contact lens insertion, epithelial alterations took place. He came to the conclusion that the severity of cytological alterations increased with lens wear time and is greater among symptomatic contact lens wearers.[54]

In 1995, a National Eye Institute/industry workshop was held, and a consensus was reached on definition, diagnosis, and therapy.[55]

In 1991, P Reddy examined the cytological alterations in 30 patients who had the dry eye condition. Conjunctival biopsy results and Impression cytology results are compared, and the results show that Impression cytology delivers the same information as biopsy. We can therefore draw the conclusion that non-invasive impression cytology can take the place of traditional biopsy in the diagnosis of dry eye disorders.[56]

MATERIALS

OUTPATIENT DEPARTMENT -

The study has been conducted in patients with Dry Eyes and in age and sex matched individuals without dry eyes who attended the Department of ophthalmology, Santosh Medical College and Hospital, Ghaziabad,UP.

STUDY SETTING-

Department of ophthalmology, Santosh Medical college and Hospital, Ghaziabad,UP

STUDY DESIGN-

Cross sectional analytical study

STUDY PERIOD-

April 2021 to October 2022

STUDY UNIT-

10-70 yrs

CONSENT & APPROVAL-

Ethical approval will be taken from the institutional ethical committee.

SAMPLE SIZE-

69 patients has come to be the sample size ,thus 40 cases (Group Ⅰ) and 40 controls (Group Ⅱ) are studied and thus a total 160 eyes examined.

C=0.5Ln(1+r/1-r)
n=[Zalpha+Zbeta/C]2+3
r= coorelation value between two variables i.e 0.38
Zalpha = 1.96 at 5% level of significance
Zbeta =1.28 at 90% power of study
n=69

INCLUSION CRITERIA:

Those patients who presented to Department of Ophthalmology with symptoms of dry eyes and positive for any one of following tests were included in group Ⅰ.
1)schirmers test(<10mm in 5min)
2)Tear film break up test(<10sec)
3)Rose Bengal staining – positive.

And age and sex matched population without symptoms of dry eyes and negative for tests mentioned above were included in group Ⅱ.

1)schirmers test(>10mm in 5min)
2)Tear film break up test(>10sec)
3)Rose Bengal staining – negative.

EXCLUSION CRITERIA:

1)Acute conjunctivitis.
2)Diagnosed case of dry eye already on treatment.
3)History of any past ocular surgery.

STUDY METHODOLOGY

During the above said period ,individuals satisfying the inclusion criteria were selected among the patients presented to Santosh Medical College and Hospital,Ghaziabad,UP. All individuals provided written consent after being fully informed.

Due to the lack of standardised diagnostic criteria at this time, the clinical diagnosis of dry eye disease is not straightforward or systematic. To evaluate symptoms and clinical indicators, several different questionnaire and diagnostic test combinations have been utilised.[57]

Due to the fact that some patients have substantial symptoms but few findings, while others have considerable clinical findings but only minor symptoms, it has been discovered that the combination of the history and examination is more effective and dependable.[58,59,60]

The subjective character of symptoms, potential neuronal hypersensitivity in recent cases, corneal hypersthesia, and diminished sensation in more severe and chronic diseases could all contribute to these changes in symptoms and clear signs.

HISTORY

The principal indicators of this condition are subjective dry eye symptoms. Burning, foreign body sensation, stinging, dryness, itching, pain, photophobia, heaviness of the lids, and ocular strain are only a few possible presenting methods. Patients who suffer from dry eyes often experience similar symptoms.

One significant pattern is the worsening of symptoms while or after engaging in certain activities or environments,[57] such as prolonged visual effort activities like reading or viewing television, which can worsen symptoms by slowing blinking and causing increased ocular surface drying.

There are already numerous questionnaires available for assessing the symptomatology of various diseases, as well as their various features like severity, impact on daily activities, and quality of life.[57]

Symptom questionnaires enable diagnosis screening, treatment effectiveness evaluation, and disease severity grading and are among the most reliable dry eye diagnostic tests.

Commonly used and more credible, the Ocular Surface Disease Index (OSDI) consists of 12 questions about symptoms experienced during the preceding week, their severity, and how they influence visual function and the ocular surface in response to external triggers A higher score indicates greater severity; the scale runs from 0-100. A score of 15 has a moderate specificity and sensitivity for the diagnosis, at 60% and 83%, respectively.[61]

According to the frequency of symptoms, the 12 questions in the Ocular Surface Disease Index (OSDI) should be evaluated from 4 to 0.

Observed throughout the past week:
1. Eyes that are light-sensitive?
2. Eyes feeling gritty
3. Sore/painful eyes?
4. Blurry vision ?
5. Low vision?

Have you had issues completing any of the following within the past week?
6. Reading
7. Night driving?
8.Working on a computer or an automated teller machine (ATM)?
9. Seeing TV?

Did you feel uneasy in any of the following conditions ver the past week?
10. Windy circumstances?
11. Areas that are extremely arid and low in humidity?
12. In air-conditioned spaces?

On a scale from 0 to 100, the OSDI Score is calculated, with higher values denoting greater disability. The index shows specificity and sensitivity in identifying patients with dry eye syndrome from normal people. The OSDI is a reliable and valid tool for assessing dry eye.
Clinical evaluation was carried out by using a proforma to grade the symptoms of dry eyes, such as burning, foreign body sensation, and photophobia, according to the OSDI Score.

Based on the graph provided in the proforma, OSDI scores were categorised into 4 (normal, mild, moderate, and severe) grading categories.

Slit lamp biomicroscopy was used to evaluate the anterior segment to check for opacities, xerosis, SPKs, debris, filaments, epithelial abnormalities, meibomitis, and sterile corneal ulcers.

SCHIRMER TEST

A popular, affordable, and simple clinical test called the Schirmer test is used to qualitatively assess tear production. Otto Schirmer first described the Schirmers test in 1903. It is still the approach that clinicians employ the most frequently to gauge aqueous tear production, according to Kashkouli et al in 2010.[62]
Many changes were made later, such as the 1953 switch by De Roetth [63] to Whatman standard No. 41 filter paper. Halberg and Behrens [64] initially released the standardised Schirmer test strips in 1961.

Whatman number 41 strip, 5 mm wide by 35 mm long, was inserted into lower eyelid for this test. A well-standardized test called the ST without anaesthesia or basal schirmers test assesses the basal tear secretion caused by conjunctival-lacrimal trigeminal reflex. [65,66]

(FIGURE-1) Whatman number 41 strip - Schirmer strips

Schirmer's test was performed with out topical anaesthesia.The strip was placed between medial 2/3rd and lateral 1/3rd of lower lid margin and asked the patient to keep the eye closed. Results were noted after 5 minutes. The strip's rate of wetting from the tears was measured in millimetres per five seconds. The test was evaluated based on grading- 4 grades (1- variable, 2-</= 10, 3-</=5,4-</=2).

(FIGURE-2) Schirmer test with Whatman No. 41 strip

(FIGURE-3) Reading of schirmer test after 5 minutes

TEAR FILM BREAK UP TIME(TBUT)

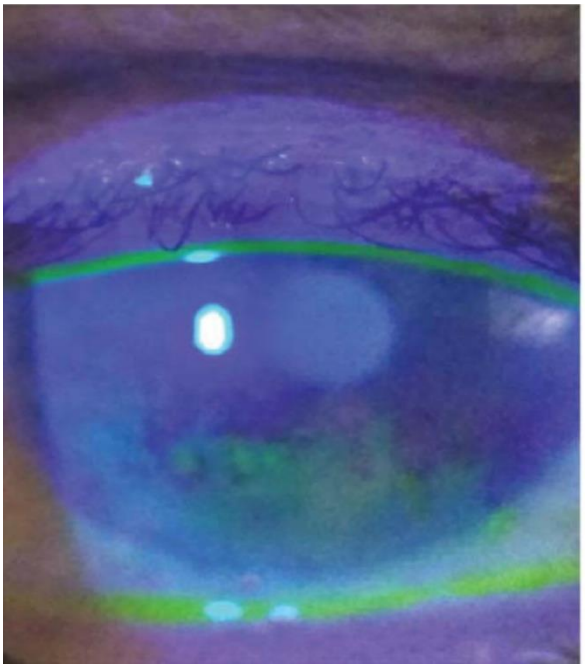
Tear break-up time (TBUT) is common, cheap and easy to perform. Tear breakup time test is the standard clinical procedure that was introduced by Norn and its high sensitivity suggests a strong connection to the dry eye.[67]

Tear stability describes the effectiveness of the cohesive forces present between the three layers of the tear film. When one or more of the layers break up, the tear film will be unstable. Fluorescein 1 mg strips were used to stain the subjects' eyes, and they were instructed not to blink.TBUT is the time interval between a complete blink and the first appearance of a dry spot in the precorneal tear film after the installation of fluorescein viewed with a cobalt blue filter.[68,69]The grading of TBUT is as follows- (1-variable, 2-</=10, 3-</= 5, 4-immediately)

(FIGURE-4) 1mg Fluroscein staining strip

(FIGURE-5) Staining inferior bulbar conjunctiva to perform TBUT

(FIGURE-6) Performing TBUT on slit lamp with blue light filter



(FIGURE-7) Noting the time for first dry spot appearance for TBUT

ROSE BENGAL STAIN

The superficial ocular surface can be evaluated using dyes to find damage that may be present in dry eye disease. These colours include rose Bengal, lissamine green, and fluorescein. These tests are inexpensive and simple to administer clinically.

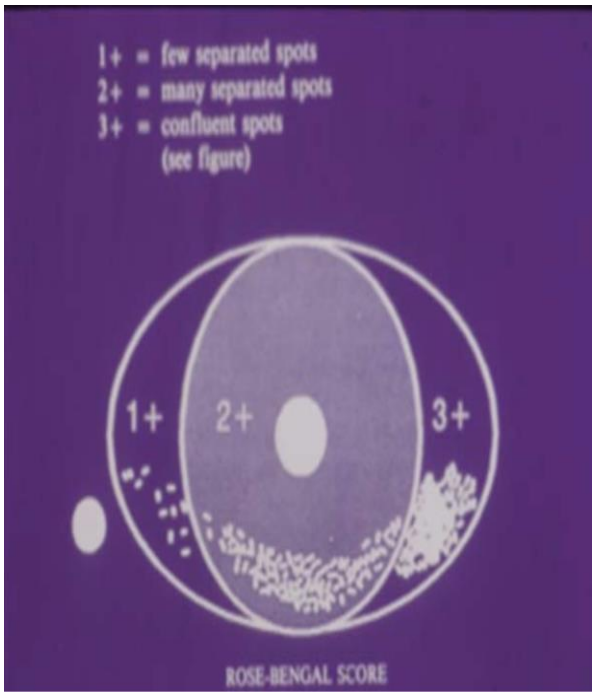
Where there is disruption in the intercellular connections, fluorescein dye will be absorbed by the corneal and conjunctival tissue. The superficial punctate keratitis is one of the common corneal stains associated with dry eyes. Unfortunately, a number of other illnesses can lead to superficial punctate keratitis hence this technique is not very accurate.[69]

By staining areas that are not completely covered by mucus, Lissamine Green and Rose Bengal are used to examine the conjunctival surface. Due to its potential for toxicity to the epithelium and ocular irritation, rose bengal must be applied under anaesthesia. This restriction does not apply to lissamine green. Oxford 80[70] and van Bijsterveld are two popular grading systems that are frequently used in clinical trials.[71]

1 % liquid rose bengal is instilled into the eye. The examiner uses green filter to assess the amount of staining on slit lamp biomicroscopy. Intensity scored in 2 exposed conjunctival zones (nasal and temporal conjunctiva) and cornea Score. The staining pattern was documented, and the samples were assessed using Van Bijsterveld's scale 0–3, a maximum of 9 score. Scores under 4 are considered normal, and those over 4 are considered abnormal (American-Europe criteria).[71,72]

(FIGURE-8) Rose Bengal staining strips

(FIGURE-9) Rose Bengal staining on conjunctiva and cornea



(FIGURE-10) Van bijsterveld score

Clinical severity was classified into four gradings: mild, moderate, severe, and extremely severe using the Dry Eye Severity Grading Scheme with Proforma (Delphi Approach to Treatment Recommendations).[59]

TABLE-1(Dry Eye Severity Grading Scheme)

Severity of dry eye	**1**	**2**	**3**	**4**
Pain, intensity, and occurrence	happens when there is environmental stress; mild and/or episodic	Stress of any kind, whether mild, chronic, or nonexistent	severe, continuous, or persistent without stress	severe, incapacitating, and/or ongoing
visible signs	No or only occasional minor tiredness	Interfering with or inhibiting activity	unpleasant, continuous, or limited activity	persistent and/or potentially disable
Congestion of conjunctiva	No congestion or mild congestion	No congestion or mild congestion	+/-	+/++
Signs of cornea and tear film	No or mild signs	Some debris, low or high meniscus	Increased tear debris, filamentory keratitis, mucus agglutination.	Increased tear debris,filamentory keratitis ,mucus agglutination , ulceration.
Meibomian/ lid glands	MGD variably present	MGD variably present	Frequency	Symblepharon, trichisis, keratinisations
Tear break up time (sec)	Variable	</=10	</= 5	Immediate
Schirmer score(mm/5 min)	Variable	</= 10	</=5	</= 2

CONJUNCTIVAL IMPRESSION CYTOLOGY

Impression cytology is the process of removing the outermost layers of the ocular surface epithelium using a cellulose acetate filter applied to the eye. The histological, immunohistological, or molecular examination of these cells is then possible.

This minimally invasive technique for examining conjunctival goblet cells was first introduced by Egbert et al.[15] Since then, the method has been utilised to assess a number of ocular surface conditions, and the original method has undergone modifications.

It is non-invasive, simple to use, and produces accurate results regarding the area tested while causing the patient the least amount of discomfort. This makes it an important tool for comprehending ocular surface diseases.

Implementations of impression cytology include the etiological diagnostic test of numerous ocular surface disorders, describing sequential variations in the conjunctival and corneal outer layer over time, surveilling clinical outcomes and staging conjunctival squamous metaplasia, as well as as an investigational tool for analysing ocular surface disease with immunostaining and DNA analysis.[16]

HISTORY

Conjunctival samples were collected by Egbert et al using Millipore filters, and they were afterwards air dried and stained with periodic acid Schiff (PAS) and hematoxylin.[15]

Tseng changed the procedure for collecting specimens and stained them using PAS and Papanicolaou stains.[16]

Maskin and Bode have described a method for using impression cytology to collect conjunctival epithelial cells for electron microscopy research.[17]

To enable immunocytochemical staining, pure nitrocellulose membranes and Biopore membrane apparatus have been utilised.[73,74]

SAMPLE COLLECTION-

The majority of authors utilise surfactant-free filter paper with pores ranging from 0.22 mm to 0.44 mm.[75] Millipore's cellulose acetate filter paper, which is trimmed into a 5 mm strip with one square end and one tapered end, is used in Tseng's modified method of collecting

specimens.[16] The pointed tip and asymmetrical form make it easier to hold and move the paper to the right location while using forceps with blunt, smooth edges.

All subjects underwent cytology in three phases.
A-Sample collection
B-Staining
C-Grading of Cytology

MATERIAL USED-

1.Filter paper made of cellulose acetate
2. Isopropyl alcohol
3. H&E stain (Hematoxylin and eosin)
4. Periodic acid schiff stain
6. Water (distilled)
7. Xylene
8. Dishwasher stains
9. Slides tray
10. Glass slides
11. Cover slips

STEPS-

-A 13 mm-diameter piece of Millipore paper cut in half in a "D" form.
- For alignment, the end of the paper that will be adhered to the nasal side is trimmed.
-Excessive tear fluid and medication are removed, and one drop of local anaesthetic is injected into the eye.
-The paper is placed over the cornea, bulbar conjunctiva or both with aseptic precautions while straddling the limbus. The underlying pathology determines the area that needs to be sampled.
-After about 5 to 10 seconds of staying in contact with the eye while putting gentle pressure, the paper is removed using forceps.

-It is essential that the lids are kept apart from the paper during the period of contact and that it is not permitted to be wetted by tear fluid, which may occasionally arise as a result of stimulation of lacrimation.

- Cell yield will be low if the paper becomes very saturated.

-The tissue is placed on a glass slide before being placed in a fixative solution-filled Koplin jar.

The generally utilised stains for routine histological staining of impression cytology specimens are papanicolaou or haematoxylin and PAS stains.

PAS STAINING-

Principle-

Amylase breaks down glycogen.

Amylase, a component of commercially available diastase, can be used to break down the glycogen in tissue sections.

REAGENTS:

1) 50 ml of a 1% aqueous solution of periodic acid that is stored at room temperature
2) 50ml of Mayers hematoxylin, kept at room temperature
3) Schiff's reagent, 10 * 5 ml, stored at 2 to 8 degrees Celsius

STAINING:

To show goblet cells, Periodic Acid- Schiff (PAS) was used to stain all of the slides with hematoxylin and eosin.

STEPS OF HEMATOXYLIN AND EOSIN STAIN

1) Give a 5-minute haematoxylin treatment.
2) Wash and soak in water for five minutes until turns blue.
3) Differentiate for 5 seconds in 1% acid alcohol.
4) Wash for 10 minutes under running water.

5) Let the 1% eosin sit for two minutes.

6) Wash for 1 to 5 minutes under running water.

7) Let the mount dry.

STEPS OF PERIODIC ACID SCHIFF STAINING:

1) Fixative was removed from the slides.

2) Oxidize for five minutes in a 1% solution of periodic acid.

3) Use distilled water to rinse.

4) Apply the Schiff reagent for five minutes.

5) Wash for five minutes in running water.

6) For 10 minutes, stain the nuclei with Mayers haematoxylin.

7) Use water to wash.

8) Mount and dehydrate.

GRADING:

Nelson's Grading was used to grade the conjunctival impression cytology.[26]

Grade 0 –

The epithelial cells are tiny and rounded. Large nuclei are present. The goblet cells are thick, rounded, and numerous.

Grade 1-

The epithelial cells are slightly bigger and more oblong. The nuclei are more compact. Despite having fewer of them, the goblet cells continue to have an oval shape and are still plump.

Grade 2 –

Larger, more polygonal, and occasionally multinucleated epithelial cells are present. The nuclei are very relatively small. The goblet cells are far fewer, smaller, and have distinct cellular boundaries.

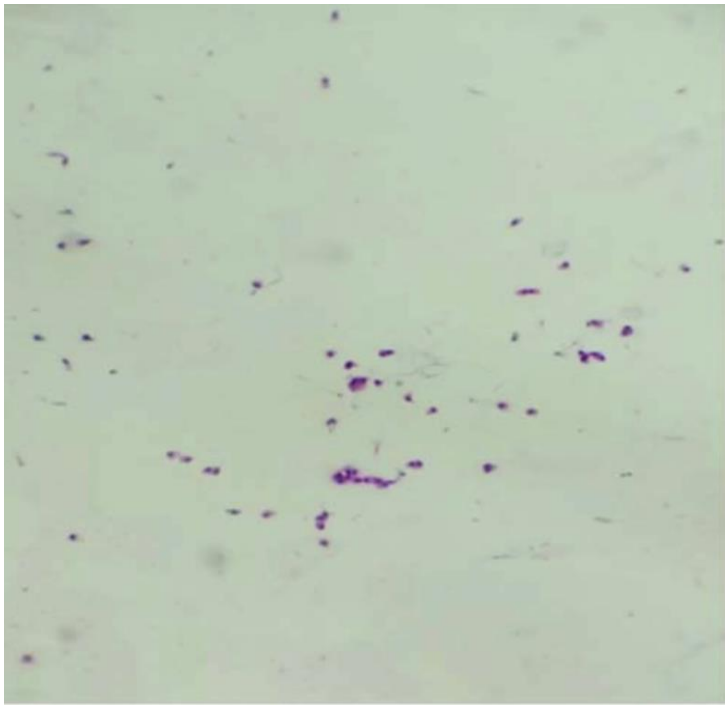
Grade 3 –

The nuclei are tiny and pyknotic, and the epithelial cells are big and more polygonal. There are no goblet cells at all.

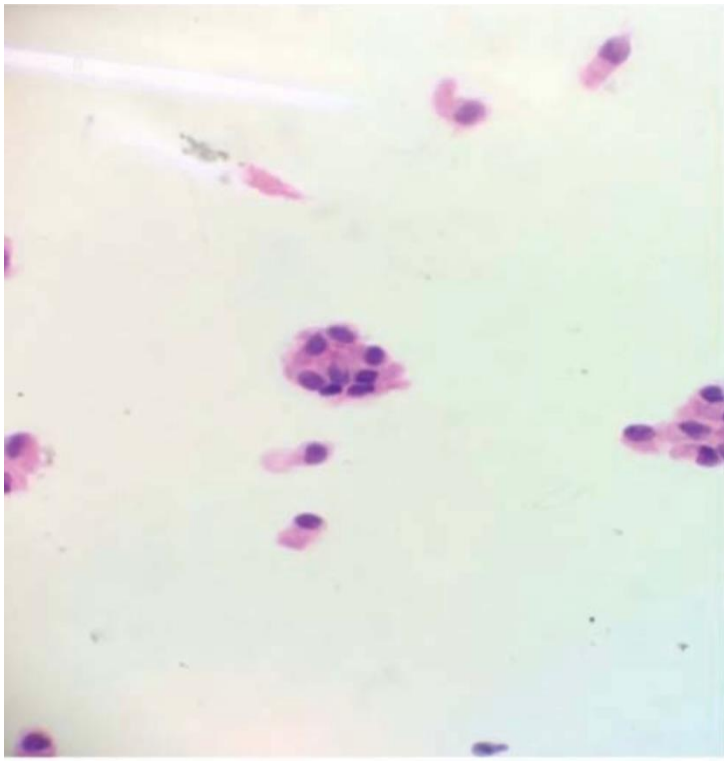
Grades 0 and 1 were considered normal whereas grades 2 and 3 were considered to have aberrant cytology.

(FIGURE-11) Sample collection for conjunctival impression cytology

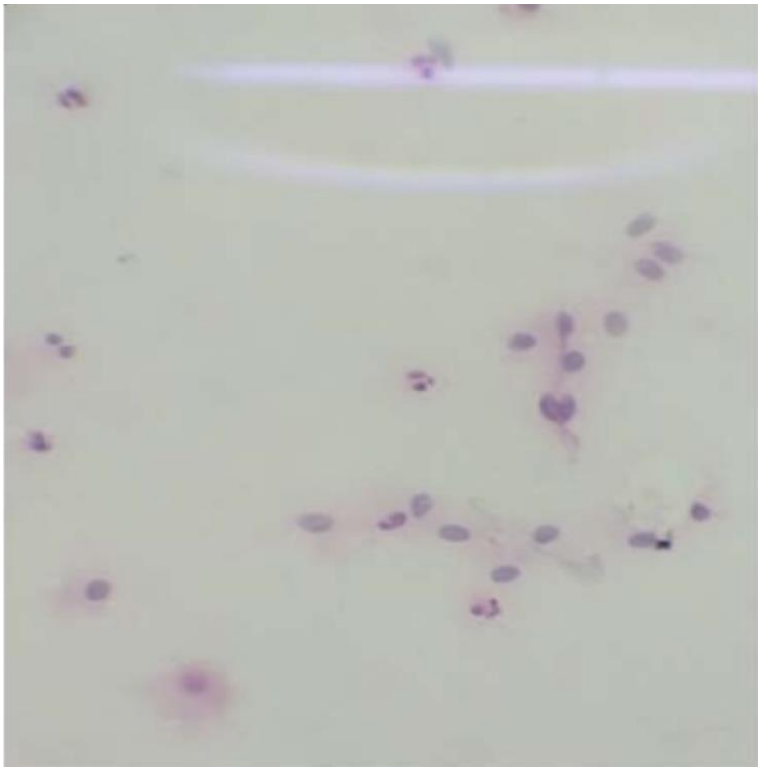
(FIGURE-12) Viewing the sample slides under microscope after PAS and H&E staining



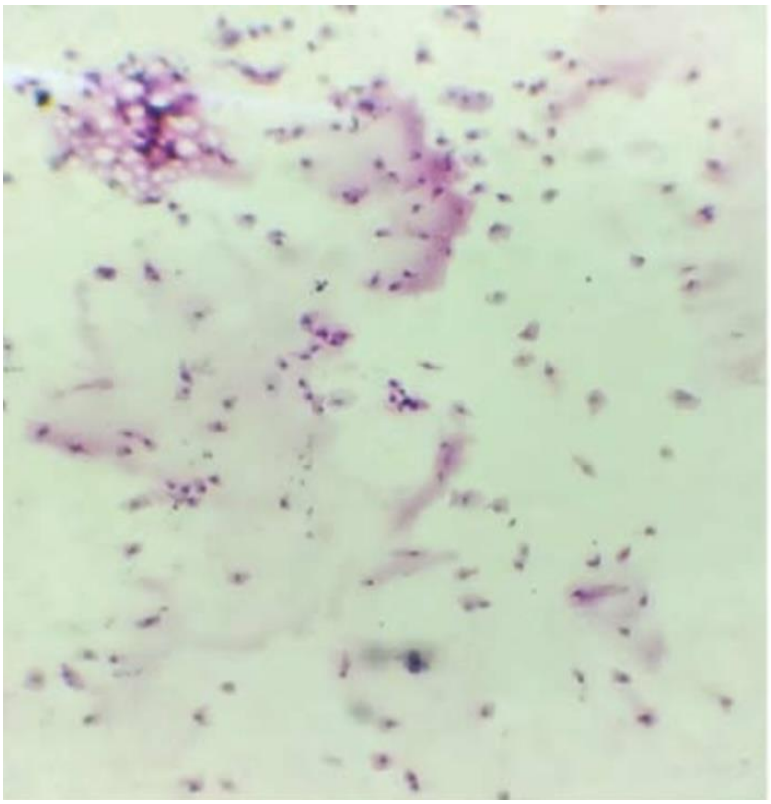
(FIGURE-13) NELSON CIC grade-0 in 10x power (low power)



(FIGURE-14 NELSON CIC grade-0 in 40x power (high power)



(FIGURE-15) NELSON CIC grade-1 in 40x power (high power)



(FIGURE-16) NELSON CIC grade-2 in 10x power (low power)

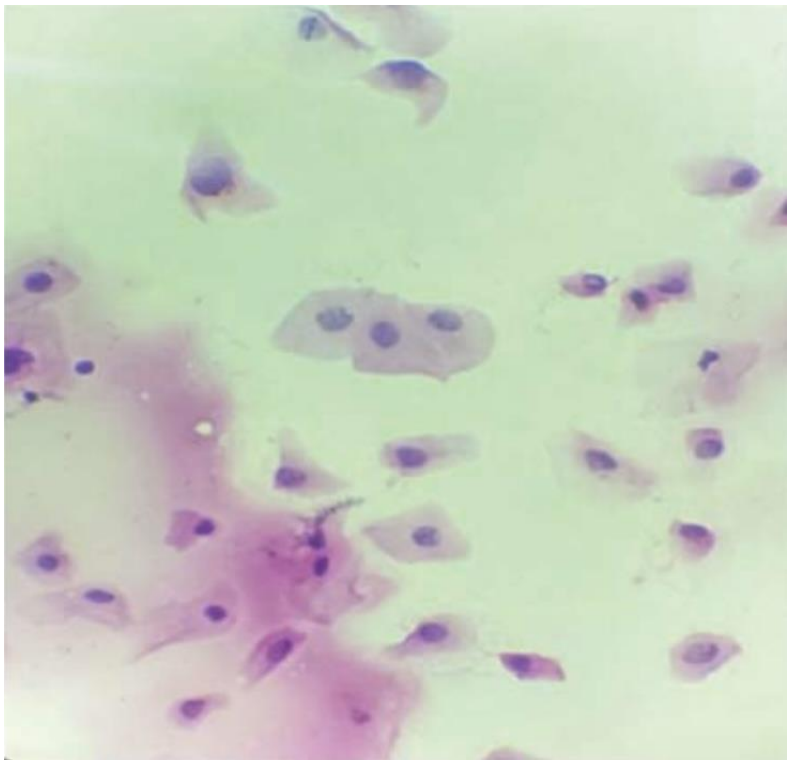
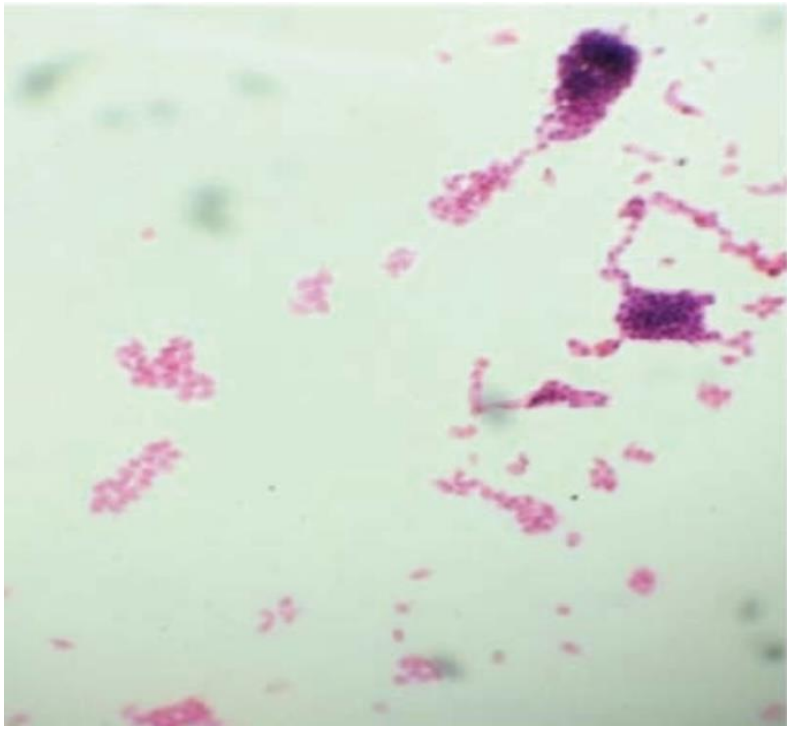
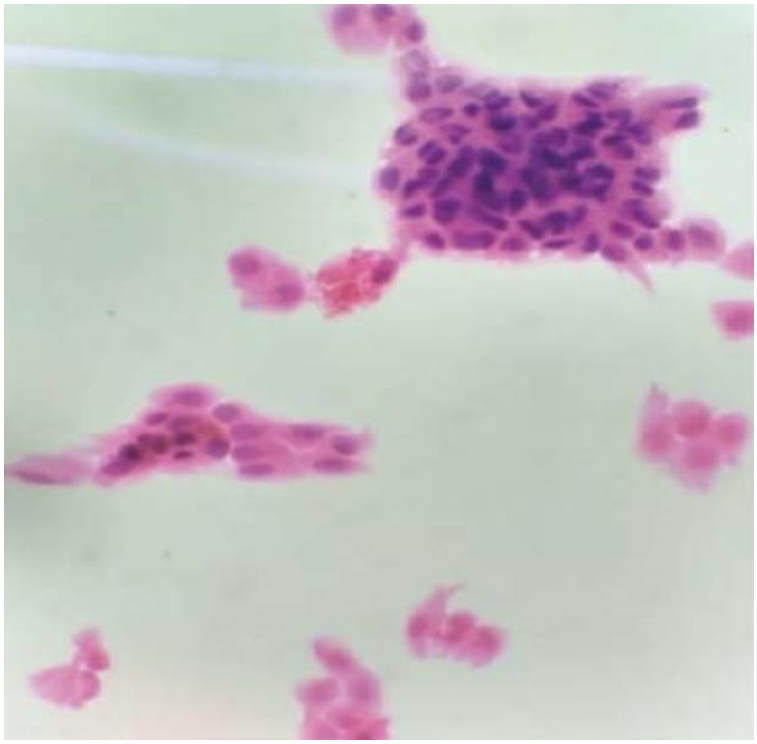


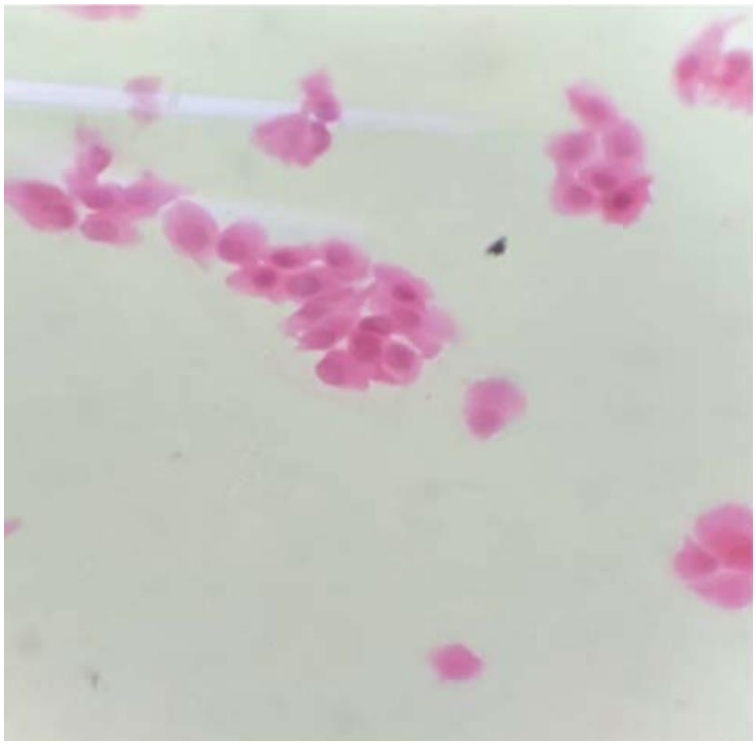
FIGURE-(17) NELSON CIC grade-2 in 40x power (high power)



(FIGURE-18) NELSON CIC grade-3 in 10x power (low power)



(FIGURE-19) NELSON CIC grade-3 in 40x power (high power-1)



(FIGURE-20) NELSON CIC grade-3 in 40x power (high power-2)

MICROSCOPY AND CLINICAL APPLICATIONS:

A flat mount or cross section preparation of the ocular surface yields more information than an impression cytology, which typically removes only 1-3 cell layers. Therefore, rather than the basal epithelium or the basement membrane, it is perfect for investigating the surface epithelium.

Egbert originally applied this technique morphologically to ascertain the density of goblet cells in various conjunctival regions.[15] The nasal palpebral conjunctiva had the highest density of goblet cells, while the temporal and palpebral conjunctiva, bulbar conjunctiva at the fornices, and bulbar conjunctiva near the limbus all had decreasing densities.[15] Additionally, impression cytology has been used to assess ocular surface conditions such keratoconjunctivitis sicca[25,26], vitamin A insufficiency[27], atopic skin disease[77] ,cicatricial pemphigoid[78] ,Vernal keratoconjunctivitis[79] , Superior limbic keratoconjunctivitis and Mucopolysaccharidoses[17], and the impact of various therapy on these.
Tseng divided conjunctival squamous metaplasia into six stages based on goblet cell density, density of goblet cells, and morphological alterations of the nucleus, nucleuscytoplasm ratios, cytoplasmic metachromatic colour changes, and the appearance of keratinization.[16]

The OSDI score, Schirmer's test grade, tear break-up time (TBUT) grade, Van Bijsterveld grade, and dry eye severity grade have all been correlated with this cytological grading.
All comparison criteria were entered into the master chart.

STATISTICAL ANALYSIS:

For statistical analysis, all data were entered into a computer data base using latest version of Microsoft excel,data analysed using appropriate tests. To examine statistical significance, chi square tests were used. (A p value of 0.05 or higher is deemed significant) and a graphical representation of the variable is shown to understand the results clearly and to measure the association for categorical dataset was analysed using appropriate tests.

RESULTS

TABLE – 2 (CASES OF DRY EYES ACCORDING TO AGE)

AGE (in yrs.)	11-20	21-30	31-40	41-50	51-60	61-70	TOTAL
CASES HAVING DRY EYES	3	6	3	7	13	8	40
% of CASES HAVING DRY EYES	7.5	15	7.5	17.5	32.5	20	100

Among 40 cases with dry eyes, maximum patients were in the age group of 51-60 years (32.5%) and minimum in the age group of 11-20 years (7.5%) and 31-40 years (7.5%). 30% cases were below the age of 40 years and 70% cases were above the age group of 40 years.

GRAPH-1 (CASES OF DRY EYES ACCORDING TO AGE)

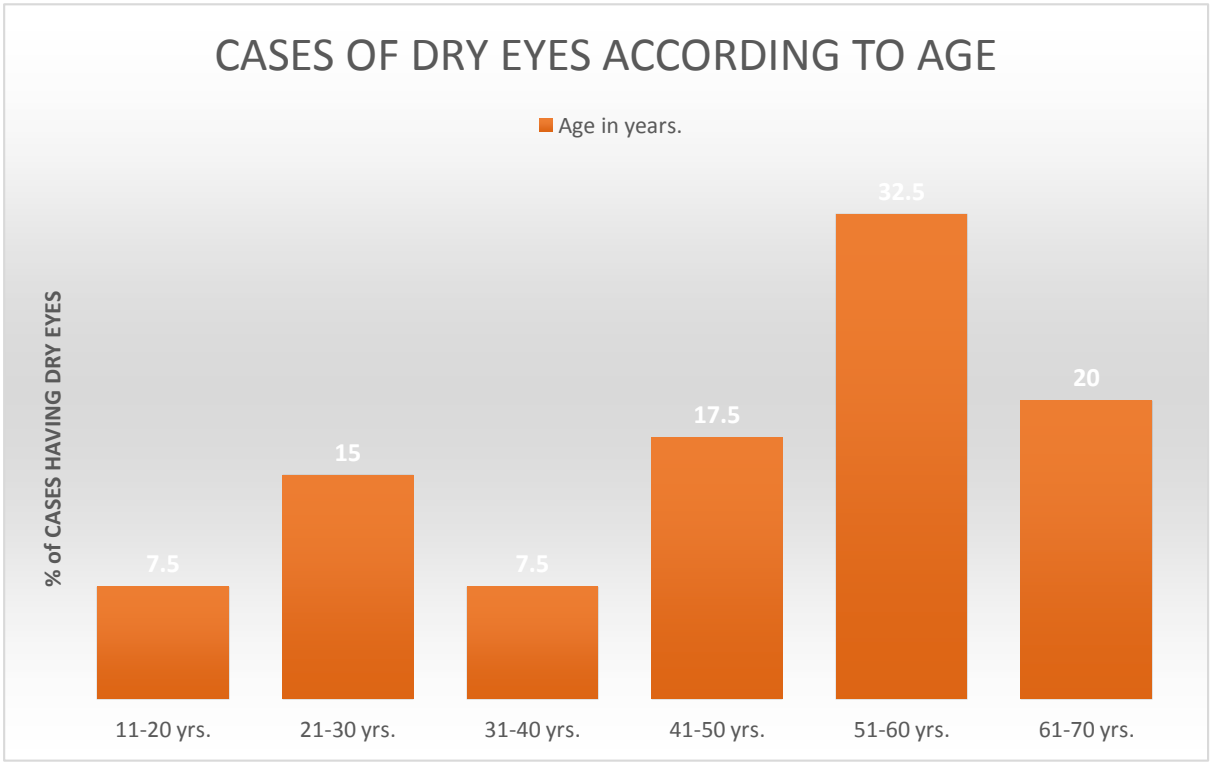


TABLE-3 (GENDER DISTRIBUTION ACCORDING TO AGE)

AGE IN YEARS	MALE	FEMALE	TOTAL
11-20 yrs.	2	1	3
21-30 yrs.	3	3	6
31-40 yrs.	2	1	3
41-50 yrs.	3	4	7
51-60 yrs.	7	6	13
61-70 yrs.	4	4	8
TOTAL	21	19	40

In the present study, amongst 40 cases with dry eyes, maximum males were from the age group of 51-60 years (33.3 %) and minimum from 31-40 years (9.5%) while maximum females were from the age group of 51-60 years (31.57%) and minimum were equally from 11-20 years (5.2%) and 31-40 years (5.2%).And cases affected with dry eye were more males (52.5%)than females (47.5%).

GRAPH-2 (GENDER DISTRIBUTION ACCORDING TO AGE)

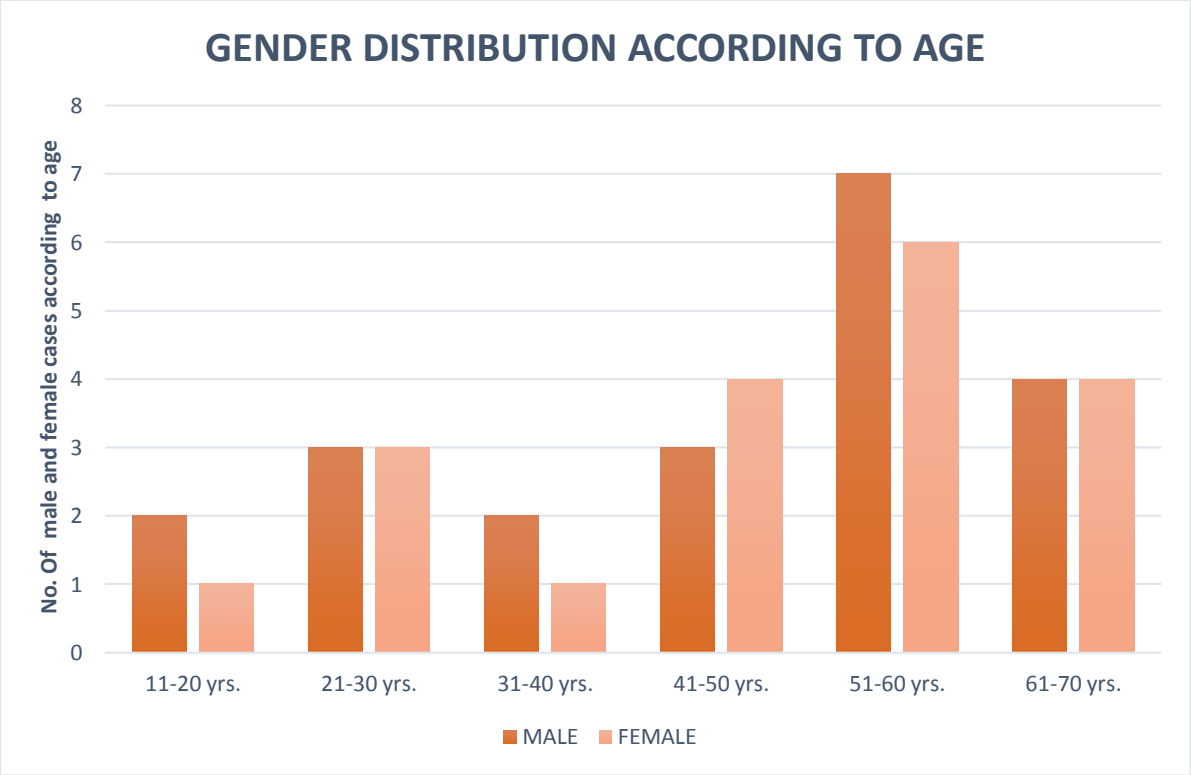


TABLE-4 (GENDER FREQUENCY)

	MALE	FEMALE	TOTAL
CASES HAVING DRY EYES	21	19	40
%	52.5	47.5	100

The study showed males 21 in number (52.5%) having dry eyes were more than females which were 19 in number (47.5%).

GRAPH-3 (GENDER FREQUENCY)

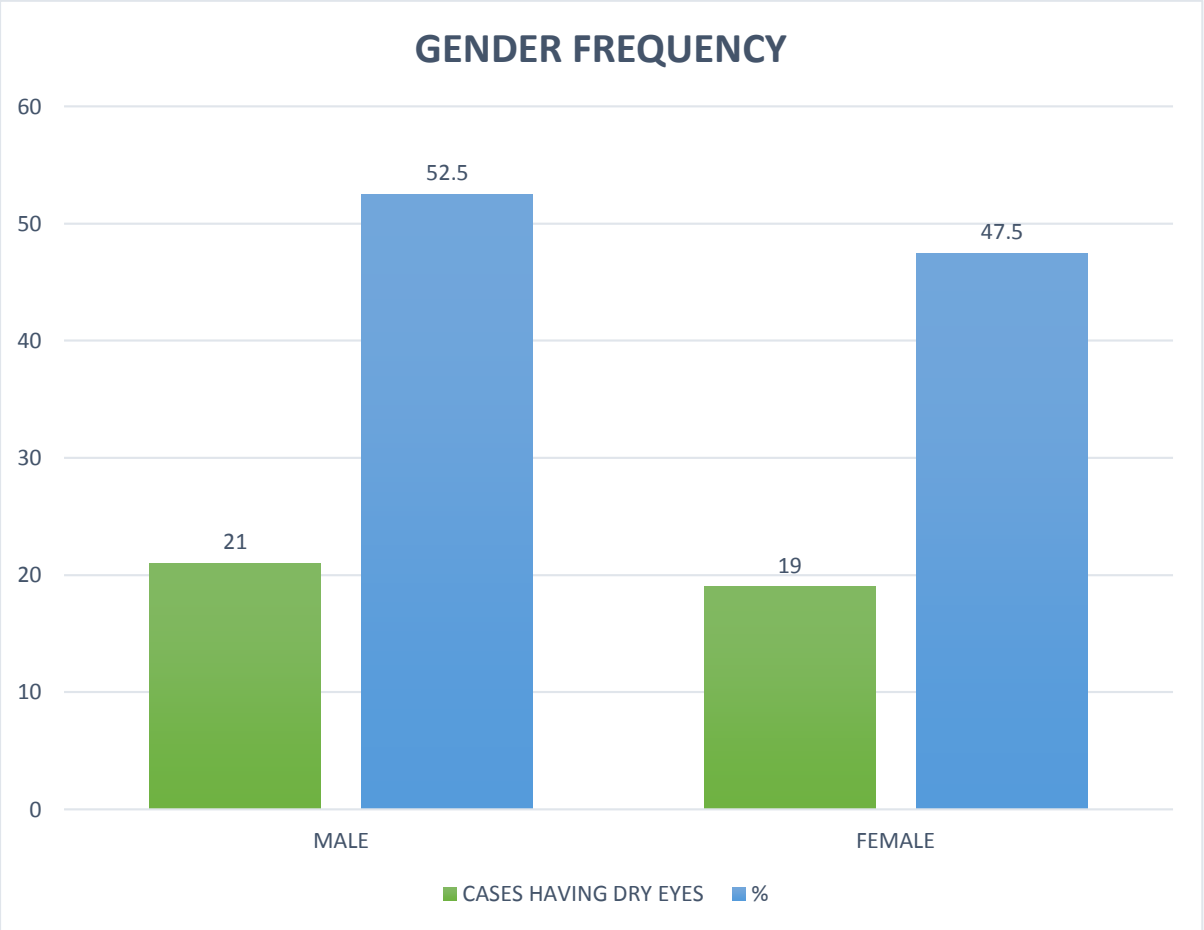


TABLE-5 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH OSDI GRADING)

GRADING OF CIC	OSDI GRADING 0(%)	1(%)	2(%)	3(%)	TOTAL
0	19(79%)	5(21%)	0(0)	0(0)	24(15%)
1	28(73.6)	5(13.1%)	5(13.1%)	0(0)	38(23.75%)
2	12(41.3)	11(37.9%)	6(20.6%)	0(0)	29(18.13%)
3	27(39.1%)	15(21.7%)	19(27.5%)	8(11.5%)	69(43.13%)
TOTAL	86(53.75%)	36(22.5%)	30(18.75%)	8(5%)	160(100%)
CHI SQUARE= 21.6, P= 0.01					

In 62 eyes with with normal cytology, 47 eyes (75.80%) had grade 0 OSDI , 10 eyes (16.1%) had grade 1 OSDI and 5 eyes (8.06%) had grade 3 OSDI and none had grade 4 OSDI.In 98 eyes with abnormal cytology, 39 eyes (39.79%) had grade 0 OSDI, 26 eyes (26.53%) had grade 1 OSDI, 25 eyes (25.5%) had grade 2 OSDI and 8 eyes (8.16%) had grade 4 OSDI. There is statistically significant correlation between OSDI AND CIC grade (chi square -21.6, p value-0.01)

GRAPH-4 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH OSDI GRADING)

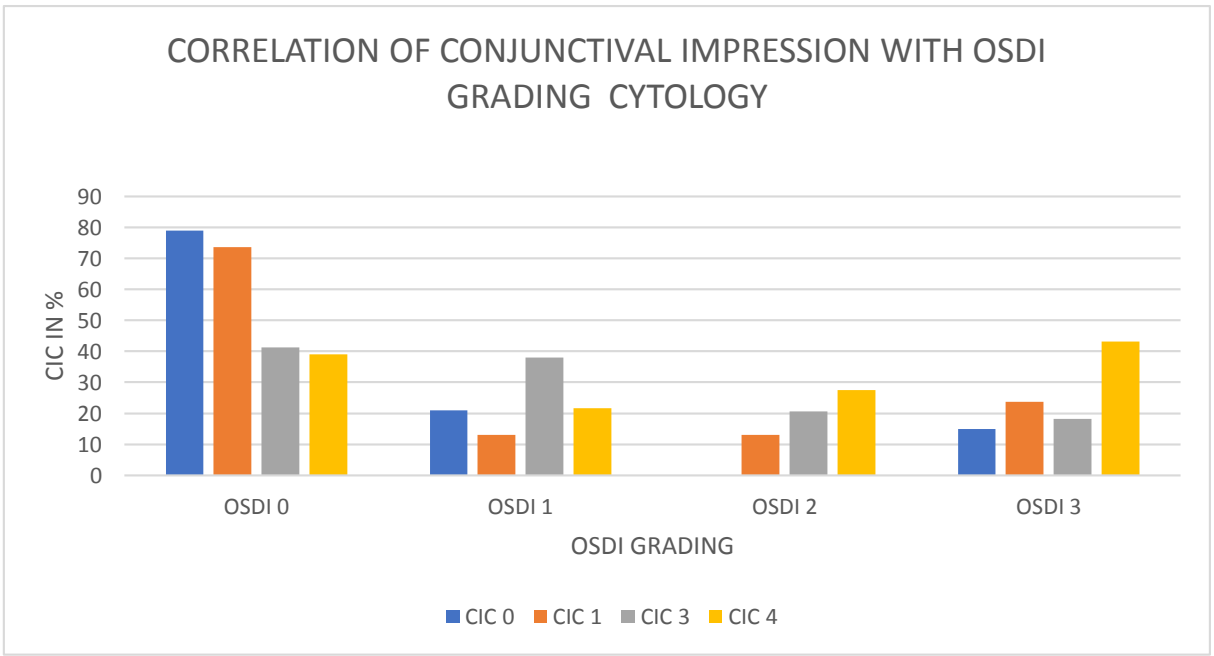


TABLE-6 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH SCHIRMERS TEST GRADING-1)

GRADING OF CIC	SCHIRMER TEST GRADING 1(%)	2(%)	3(%)	4(%)	TOTAL
0	19(79.1%)	5(20.8%)	0(0)	0(0)	24(15%)
1	33(86.8%)	4(10.5%)	1(2.6%)	0(0)	38(23.76%)
2	22(75.8%)	4(13.7%)	3(10.3%)	0(0)	29(18.13%)
3	34(49.3%)	20(29%)	15(21.7%)	0(0)	69(43.13%)
TOTAL	108(49.3%)	33(20.6%)	19(11.8%)	0(0)	160(100%)
CHI SQUARE= 15.4, P = 0.08					

In 62 eyes with with normal cytology(CIC grade 0 and 1) -52 eyes (83.8%) had ST grade 1, 9 eyes (14.5%) had ST grade 2, 1 eye (1.61%) had grade ST 3 and none had ST grade 4. In 98 eyes with abnormal cytology (CIC grade 2 and 3), 56 eyes (57.14%) had ST grade 1, 24 eyes had (24.4%) ST grade 2, 18 eyes (18.36%) had ST grade 3 and none had ST grade 4.

GRAPH-5 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH SCHIRMERS TEST GRADING-1)

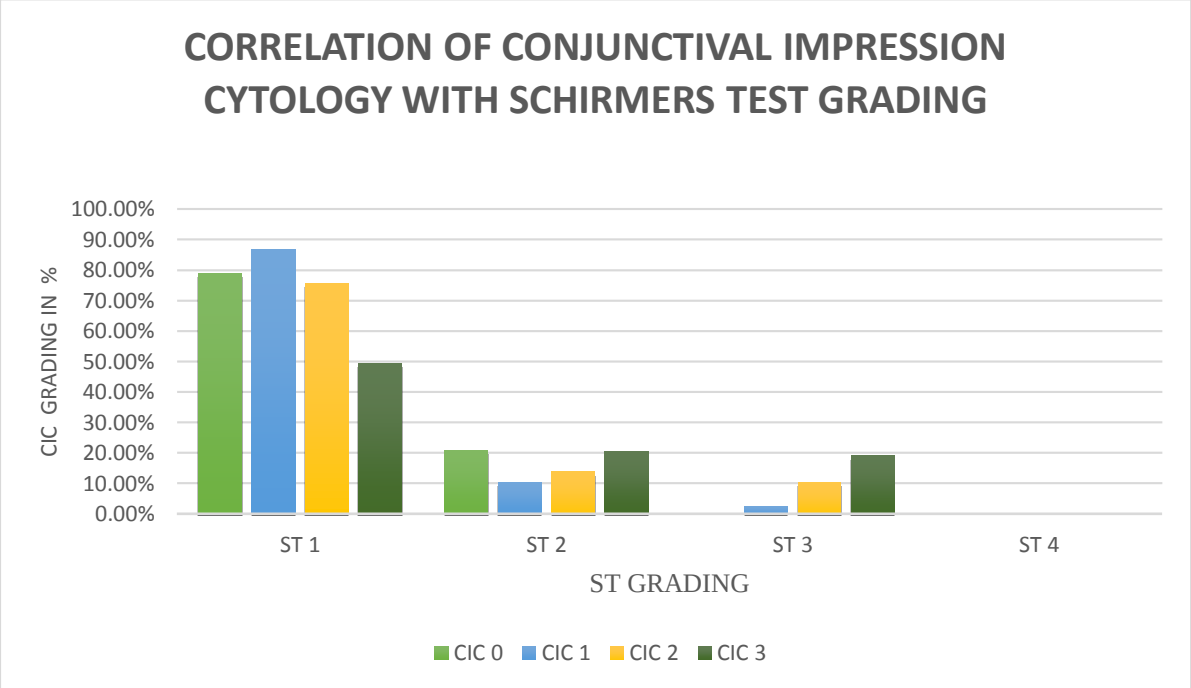


TABLE-7 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH SCHIRMERS TEST GRADING-2)

GRADING OF CIC	SCHIRMER TEST GRADING 1(%)	2(%)	3(%)	4(%)	TOTAL
NORMAL	52(48.1%)	9(27.7%)	1(5.2%)	0(0)	62 (38.75%)
ABNORMAL	56(51.85)	24(72.7)	18(14.7%)	0(0)	98 (61.25%)
TOTAL	108 (67.5%)	33 (20.6%)	19 (11.8%)	0(0)	160 (100%)
CHI SQUARE= 8.134, P= 0.043					

There is statistically significant correlation between the ST grading with that of CIC grading (chi square value- 8.134 and p value 0f 0.043)

GRAPH-6 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH SCHIRMERS TEST GRADING-2)

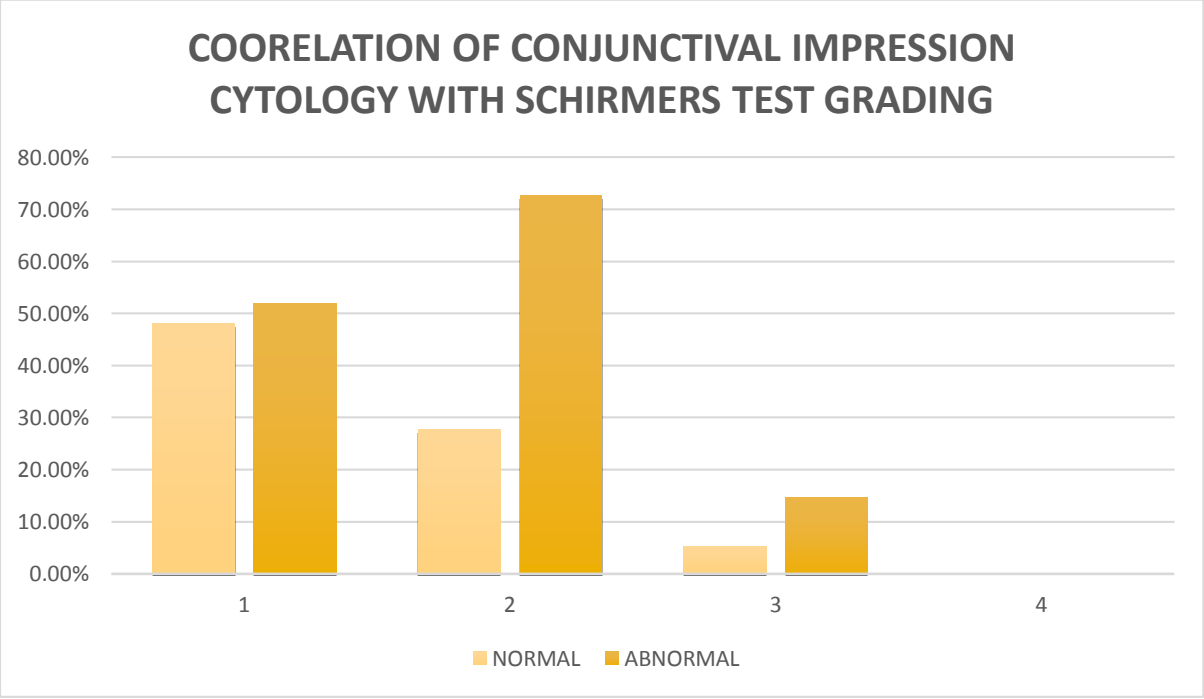


TABLE-8 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH TEAR BREAK UP TIME-1)

GRADING OF CIC	TEAR BREAK UP TIME TEST GRADING				
	1(%)	2(%)	3(%)	4(%)	TOTAL
0	19(79%)	4(16.6%)	1(4.1%)	0(0)	24(15%)
1	29(76.3%)	5(13.1%)	4(10.5%)	0(0)	38(23.75%)
2	8(27.5%)	12(41.3%)	9(31%)	0(0)	29(18.13%)
3	27(39.1%)	15(21.7%)	18(26%)	9(13%)	69(43.13%)
TOTAL	83(51.8%)	36(22.5%)	32(20%)	9(5.6%)	160(100%)
CHI SQUARE= 23.13, P= 0.006					

In 62 eyes with normal cytology (CIC grade 0 and 1), 48 eyes (77.4%) had TBUT grade 1, 9 eyes (14.5%) had TBUT grade 2, 5 eyes (8.06%) had TBUT grade 3 and none had TBUT grade 4. In 98 eyes with abnormal cytology (CIC grade 2 and 3), 35 eyes (35.7%) had TBUT grade 1, 27 eyes (27.5%) had TBUT grade 2 and 3 and 9 eyes (9.18%) had TBUT grade 4.

GRAPH-7 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH TEAR BREAK UP TIME-1)

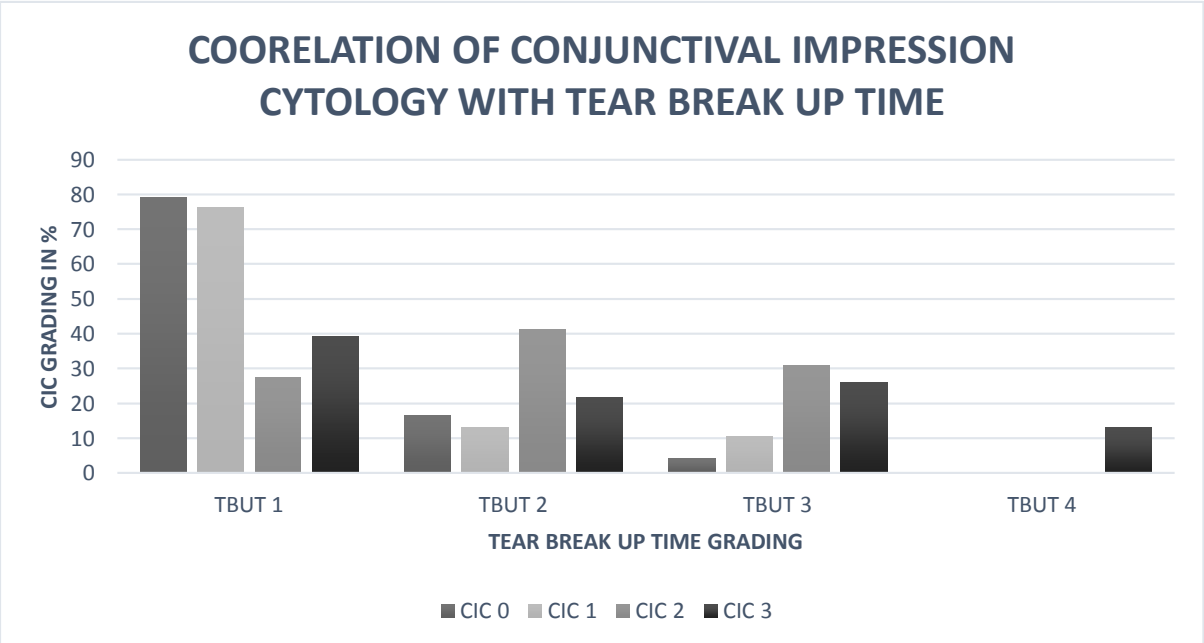


TABLE-9 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH TEAR BREAK UP TIME-2)

GRADING OF CIC	TEAR BREAK UP TIME TEST GRADING 1(%)	2(%)	3(%)	4(%) TOTAL
NORMAL	48(57.8%)	9(25%)	5(15.6%)	0(0)	62 (38.75%)
ABNORMAL	35(42.1%)	27(75%)	27(84.3%)	9(100%)	98 (61.25%)
TOTAL	83(51.8%)	36(22.5 %)	32(20%)	9(5.6%)	160 (100%)
CHI SQUARE= 12.88, P= 0.0043					

There is statistically significant correlation between the TBUT grading with that of CIC grading(chi square value- 12.88 and p value 0f 0.0043)

GRAPH-8 (CORRELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH TEAR BREAK UP TIME-2)

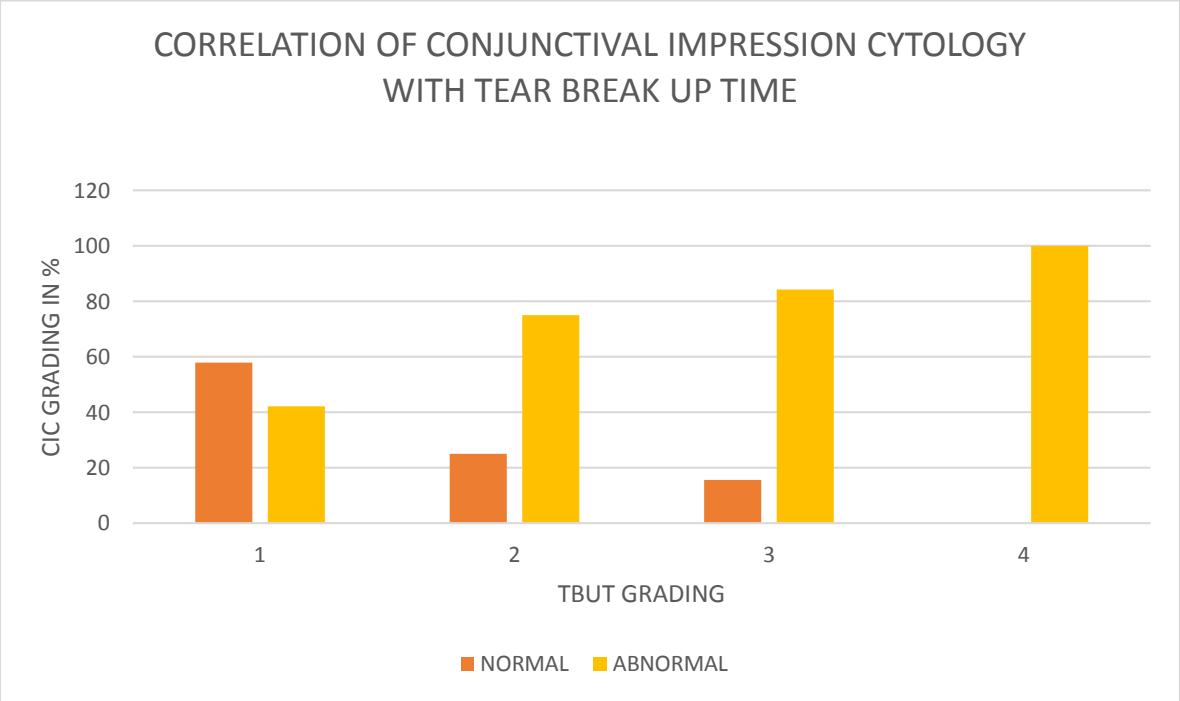


TABLE-10 (COORELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH VAN BIJSTERVELD GRADING)

GRADING OF CIC	VAN BIJSTERVELD SCORE NORMAL*	ABNORMAL *	TOTAL
0	24(100%)	0(0)	24(15%)
1	35(92.1%)	3(7.8%)	38(23.76%)
2	23(79.3%)	6(20.6%)	29(18.13%)
3	41(59.4%)	28(40.5%)	69(43.13%)
TOTAL	123(76.87%)	37(23.13%)	160(100%)
CHI SQUARE= 22.85, P= 0.000			

***NORMAL- </= 4 score of VBS**

***ABNORMAL- 4 score of VBS**

In 62 eyes with normal cytology, 59 eyes (95.16%) had normal VBS score and 3 eyes (4.8%) had abnormal VBS score and in 98 eyes with abnormal cytology, 64 eyes (65.3%) normal VBS score and 34 eyes (34.6%) had abnormal VBS score. There is statistically significant correlation between VB score and CIC grading(chi square value-22.85, p valve- 0.00).

GRAPH-9 (COORELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH VAN BIJSTERVELD GRADING)

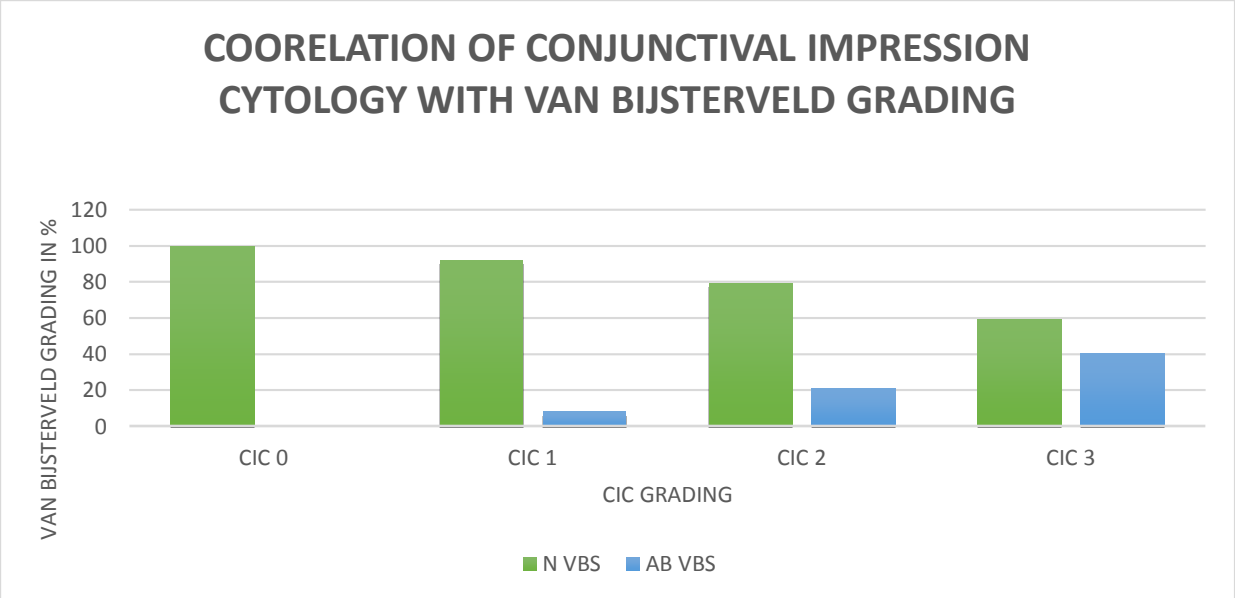


TABLE 11 (COORELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH DRY EYE SEVERITY GRADING)

GRADING OF CIC	DRY EYE SEVERITY GRADING 1(%)	2(%)	3(%)	4(%)	TOTAL(%)
0	1	5	0	0	6
1	0	5	3	2	10
2	5	11	6	0	22
3	5	15	16	6	42
TOTAL	11(13.75%)	36(45.0%)	25(31.25%)	8(10%)	80(100%)
CHI SQUARE= 8.07 , P= 0.054					

The statistically significant correlation between CIC grade and DES grading with p value(0.05)

GRAPH-10 (COORELATION OF CONJUNCTIVAL IMPRESSION CYTOLOGY WITH DRY EYE SEVERITY GRADING)

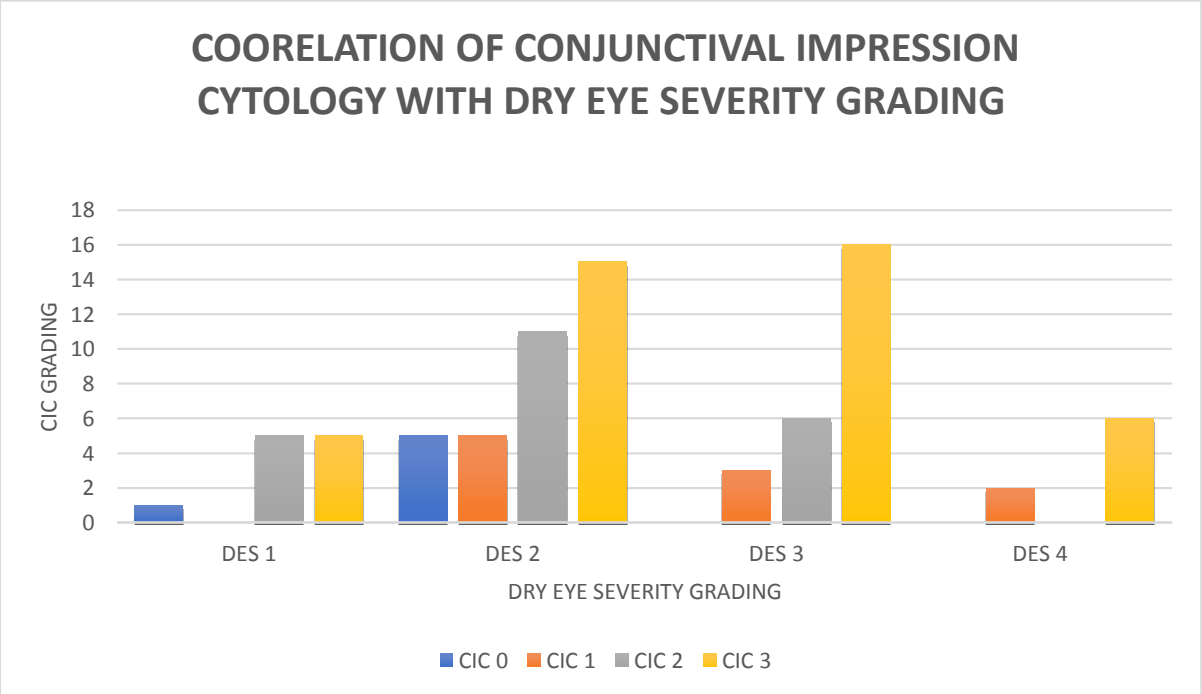
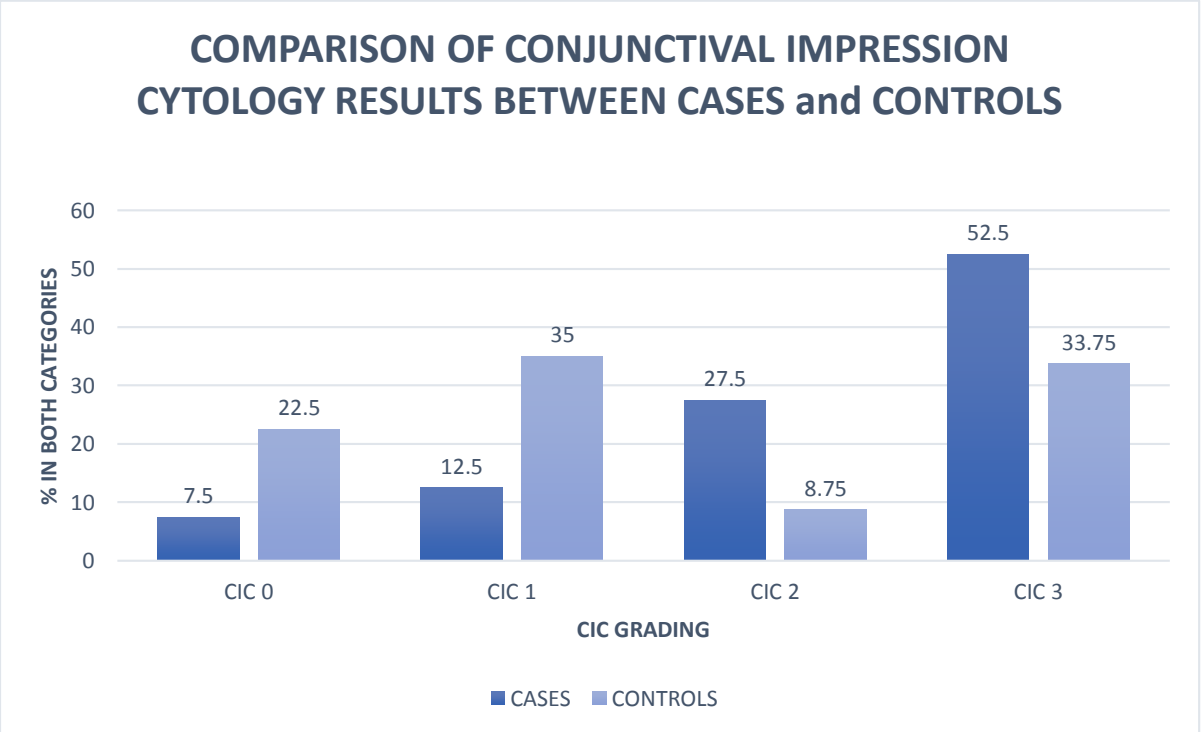


TABLE-12 COMPARISON OF CONJUNCTIVAL IMPRESSION CYTOLOGY RESULTS BETWEEN GROUP 1 (CASES) and GROUP 2 (CONTROLS)

CATEGORY	CONJUNCTIVAL IMPRESSION CYTOLOGY 0 1 2 3 TOTAL				
CASES	6(7.5%)	10(12.5%)	22(27.5%)	42(52.5%)	80(100%)
CONTROLS	18(22.5%)	28(35%)	7(8.75%)	27(33.75%)	80(100%)
CHI SQUARE VALUE - 15.46, p value- 0.001					

From 80 eyes of cases, 63 eyes (78.75%) had abnormal CIC and 17 (21.25%) eyes had normal CIC and from 80 eyes of controls, 34 eyes (42.5%) had abnormal CIC and 46 eyes (57.5%) had normal CIC. There is statistically significant correlation in cytology grading between 2 groups.(chi sqaqre value- 15.46, p value- 0.001)

GRAPH -11 COMPARISON OF CONJUNCTIVAL IMPRESSION CYTOLOGY RESULTS BETWEEN GROUP 1 (CASES) and GROUP 2 (CONTROLS)



DISCUSSION

The study contained a total of 80 individuals divided into 40 cases (Group Ⅰ) and similar age and sex matched 40 controls (Group Ⅱ) who met the specified inclusion and exclusion requirements were included in the study. Thus a total of 160 eyes were examined to evaluate To determine a correlation between clinical and cytological tests used to assess the severity of dry eye and to assess the outcomes of conjunctival impression cytology in patients with dry eye and in normal individuals .

AGE DISTRIBUTION-

In our study, participants were separated into 7 groups based on their age. Participants were sorted into eight groups by decade by Norn (1969).[80]

This sort of grouping has the benefit of allowing comparisons between the groups without regard to gender or age.[81] Additionally, the findings from a certain age group would only apply to that age group. This aids the researcher in comprehending age-related phenomena.

Among 40 cases with dry eyes, maximum patients were in the age group of 51-60 years (32.5%) and minimum in the age group of 11-20 yrs (7.5%) and 31-40 yrs (7.5%). 30% cases were below the age of 40 yrs and 70% cases were above the age group of 40 yrs.

The fact that 70% of the cases in this study were people over 40 suggests that the prevalence of dry eyes rises with age. Similar findings were made in the 2017 study by Farrand K.F., who discovered that the diagnosed DED group was older, with 72% of individuals being over 50 years old as opposed to 45% in the nonDED group.[82]

GENDER FREQUENCY-

In the present study, amongst 40 cases with dry eyes, maximum males were from the age group of 51-60 years (33.3 %) and minimum from 31-40 years (9.5%) while maximum females were from the age group of 51-60 years (31.57%) and minimum were equally from 11-20 years (5.2%) and 31-40 years (5.2%).And cases affected with dry eye were more males (52.5%)than females (47.5%).

In contrast, some research, such as the one by Farrand K.F. in 2017 revealed that women (8.8%, 11.1 million) had a higher prevalence than men (4.5%, 5.3 million). This discrepancy can be brought on by women in rural areas not seeking out medical care.[82]

COMPARISON BETWEEN CIC AND OSDI GRADING-

Higher OSDI scores (grade 2 and 3 severity) correlated better with abnormal cytology grades of CIC in the current study than lower OSDI scores (grade 0 and 1), where a large number of participants had abnormal cytology (p value –0.01).

According to Zuazo F et al., the nelsons CIC grading was substantially higher in patients with a severe grade of OSDI score (1.41±0.14) compared to healthy people (0.86±0.09) with p value(<0.01).[45]

COMPARISON BETWEEN CIC AND ST GRADING-

In the current study, normal cytology is linked to lower schirmers test grades (0 and 1), whereas abnormal cytology also significantly had lower schirmer test grades. As a result, the statistical significance had a low p value (0.043). The pathology in aqueous production may be the cause of this questionable significance, as the schirmers test mostly identified aqueous insufficiency rather than ocular surface abnormality.

According to a 2011 study by Shrestha E. et al., only 8 normal eyes with abnormal cytology had the Schirmer's value of less than 10 mm in 5 minutes, and there was a strong link between CIC and abnormal cytology in dry eyes with the Schirmer's value of less than 10 mm in 5 minutes.[47]

In a study by P Kumar et al. published in 2014, it was shown that 83.8% of normal and 52.1% of abnormal cytologies had schirmers values more than 10 mm, compared to 11.5% of normal and 27.1% of abnormal cytologies, which is comparatively different from the current study.[44]

COMPARISON OF CIC AND TBUT GRADING-

In the current study, 77.4% of normal cytology had a TBUT of greater than 10 seconds, compared to just 35.7% of abnormal cytology. Very less eyes with a grade of severe CIC had TBUTs under two seconds. The association between the CIC and TBUT grades was therefore statistically significant. (0.006 as p value)

This is similar to the Shrestha E et al study from 2011, which found that 32.0% of dry eye patients with TBUT less than 10 sec and 17.2% of those with TBUT greater than 10 sec had aberrant cytologies. 95.1 % of normal eyes exhibited TBUT lasting longer than 10 seconds, and of those, 72.9% had normal cytologies and only 22.2% had abnormal ones.[47]

P Kumar et al study's from 2014 found that TBUT ranged from 5 seconds in 11.57% of normal cytology to >10 seconds in 42.14% of aberrant cytology.[44]

COMPARISON OF CIC AND VBS OF ROSE BENGAL STAINING-

In the current study, 95.1% of patients with normal cytology had normal VB scores, compared to 65.3% of patients with abnormal cytology who had normal VBS and 34.6% who had abnormal VBS. Between the CIC and VB scores of the Rose Bengal staining, there was a statistically significant association with a p value (0.00).

In a 2014 study by P Kumar et al, it was found that rose Bengal staining had an abnormal VB score in 19.9% of normal and 80.09% of pathological cytology, compared to normal VB scores in 80.09% of normal and 19.21% of abnormal cytology.[44]

COMPARISON OF CIC GRADING WITH DRY EYE SEVERITY GRADE-

In present study, 68.75 % of normal cytology had 1 and 2 DES Grade where as 43.75% of abnormal cytology had 3 and 4 DES grading. There was statistically significant correlation between CIC grade and DES grading with p value(0.05)

COMPARISON OF CIC GRADING BETWEEN 2 GROUPS-

In the current study, 80 eyes from cases(with dry eyes) had a 20% normal cytology and 80% abnormal cytology. 80 eyes from controls (without dry eyes) had cytology that was 53.70% normal and 46.29% abnormal cytology.

In the CIC grading, there was a statistically significant correlation between the two groups, with aberrant cytology in those with dry eyes and considerable normal cytology in those without dry eyes. (chi squared value: 15.46, p = 0.001).

This is analogous to a research by Shrestha E. et al. in which aberrant cytology was found in the eyes of 23.6% of normal people and 49.2% of dry eye patients.[47]

Reddy M et al. (1991) found that 76.6% of dry eyes showed aberrant cytology, and Gautam TSK et al. (2015) found that 53.7% showed abnormal cytology.[56,83]

CONCLUSION

Results indicated that older age groups, with a peak age group between 51 and 60 years, have a higher prevalence of dry eyes. The greater prevalence of dry eyes in men in the current study can be attributed to rural women's less frequent use of health care facilities.

Comparing dry eye patients to healthy people, it has been discovered that there is a considerable ocular surface irregularity attributed with these cases. This will help to reinforce the idea that dry eyes and abnormalities of the ocular surface cause a vicious cycle of dry eye syndromes.

Conjunctival impression cytology and tear film tests including TBUT, rose Bengal staining, and other tests have been found to significantly correlate with one another, with the exception of the schirmers test, where there was only marginally significant correlation. This may indicate that the schirmers test will identify an aqueous shortage rather than the ocular surface epithelial abnormalities that is picked up better by TBUT and rose Bengal staining.

Between the OSDI Score and the grade of dry eye severity determined by conjunctival impression cytology, there was a strong association. This will imply that the latter will be used to evaluate dry eyes.

This finding indicates that conjunctival impression cytology can be effective in detecting early ocular surface abnormalities.

1) To identify ocular surface abnormalities linked to dry eyes in order to end the cycle.
2) To identify dry eyes
3) To determine the extent of dry eyes and to begin the proper course of treatment.
4) To keep track of the prognosis following treatment.

This research concludes that there is strong correlation between conjunctival impression cytology and tear function tests. Thus this non-invasive, rapid and easy investigation is valuable in assessing dry eye disease and can serve as an excellent alternative to conjunctival biopsy in diagnosing and evaluating the prognosis of dry eye disease.

SUMMARY

This study on **"THE CORRELATION OF TEAR FUNCTION TESTS AND CONJUNCTIVAL IMPRESSION CYTOLOGY IN DRY EYES AMONG PATIENTS ATTENDING SANTOSH MEDICAL HOSPITAL, GHAZIABAD"** was carried out between april 2021 and October 2022 at the Department of Ophthalmology at Santosh medical college and hospital ,Ghaziabad.

The study's goals were to evaluate the conjunctival impression cytology results between dry eye patients and healthy individuals and to demonstrate a correlation between clinical and cytological in determining the severity of dry eye**.**

A total of 80 people with dry eyes (group 1) and 80 people without dry eyes (group 2) who were matched for age and sex were investigated. Using cellulose acetate filter paper, OSDI, schirmers test, TBUT, rose bengal staining, and conjunctival impression were performed. Cytology grading and clinical grading were compared.

The results indicated that men in the 51–60 years age range had a higher prevalence of dry eyes. It was discovered that people with dry eyes had far more abnormal cytology than people without dry eyes.(p value- 0.001). Conjunctival impression cytology showed a statistically significant connection with OSDI ($p = 0.01$), Schirmers test ($p = 0.043$), TBUT ($p = 0.043$), Rose Bengal staining ($p = 0.00$), and Dry Eye Severity Grading (p value-0.05).

This research concludes that there is strong correlation between conjunctival impression cytology and tear function tests. Thus this non-invasive, rapid and easy investigation which can also be done in OPD is valuable in assessing dry eye disease and can serve as an excellent alternative to conjunctival biopsy in diagnosing and evaluating the prognosis of dry eye disease.

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ANNEXURE-1
PROFORMA

OPD NO-

Name-

Age-

Sex-

Occupation-

Address-

Chief complaints- Burning sensation

-Foreign body sensation

- Photophobia

OSDI SCORE-

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?.	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?.	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

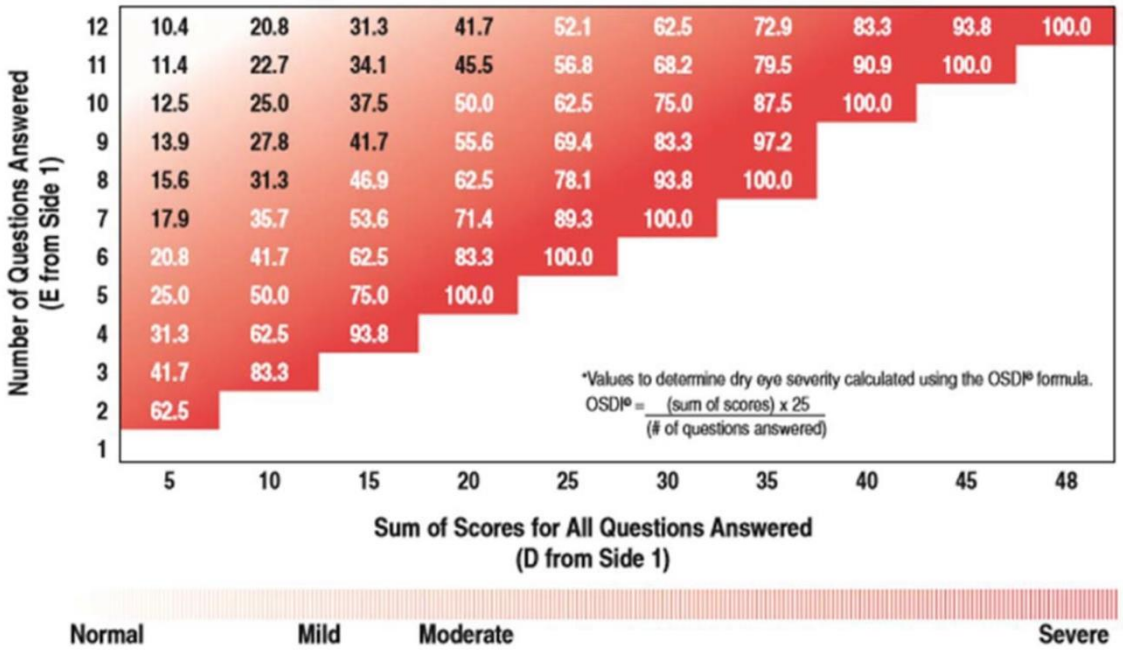
Subtotal score for answers 6 to 9 (B)

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?.	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?. . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D (D = sum of scores for all questions answered)	(D)
Total number of questions answered (do not include questions answered N/A)	(E)

ASSESSMENGT-



OSDI GRADING-

NORMAL-0

MILD-1

MODERATE-2

SEVERE-3

SCHIRMERS TEST- Right eye Left eye

Grade 1-

Grade 2-

Grade 3-

Grade 4-

SLIT LAMP EXAMINATION-

Xerosis-

SPKs, -

Debris-

Filaments-

Epithelial defects-

Meibomitis-

Sterile corneal ulcers-

Corneal thinning-

Corneal melting-

Corneal opacities-

TBUT GRADING-

Grade 1-

Grade 2-

Grade 3-

Grade 4-

ROSE BENGAL STAINING-

Van bijsterveld score-

Normal/ Abnormal VBS-

DRY EYE SEVERITY GRADING-

1- Mild-

2- Moderate-

3- Severe-

4- Very severe-

CONJUNCTIVAL IMPRESSION CYTOLOGY-

Grade 0-

Grade 1-

Grade 2-

Grade 3

ANNEXURE-2

SANTOSH MEDICAL COLLEGE AND HOSPITAL

INFORMED CONSENT

I.....S/D/W of..... Resident of..... Hereby declare that I give my full and free & voluntary informed consent in the thesis entitled **" THE CORELATION IN TEAR FUNCTION TESTS AND CONJUNCTIVAL IMPRESSION CYTOLOGY IN DRY EYE PATIENTS "**

DR. SAGARIKA RAO has informed to me to my full satisfaction, in the language I understand about the purpose and nature of the study & various physical examinations by the study to be carried out. I have been informed about the duration of the study and instructions to be followed during the study period.

I give full consent for being enrolled in the above study and I reserve my rights to withdraw from the study whenever I wish prejudice of my right to undergo further treatment at Santosh Hospital Ghaziabad U.P.

Signature of Attending. Signature Of Patient

संतोष मेडकिल कॉलेज एवं अस्पताल (गाजयिाबाद)

सूचतिसहमतर्ि

मैं _____ बेटा_____

नविासी_____ घोषणा करता हूं कमैंडॉ.__

_____, ने मुझे मेरी भाषा में अपने अध्ययन, अध्ययन के उद्देश्य के बारेमे, और अ

ध्ययन के दौरान अपना ईजानेवाली वभिन्नि प्रयोगशाला जांच के बारे में सूचति कर दयिाहै। मुझे अध्य

यन की अवधि और उसके दौरान अपना एजाने वाले नर्दिेश भी मेरी भाषा मे समझादए गए हैं| मैं उपरो

क्त अध्ययन में बनिा कसिी दबाव के दाखलिा ले रहा हूं और पूण सहमतर्ि दे रहाहूं| सन्तोष अस्पताल,

गाजयिाबाद में अपने आगेके उपचार पर बनिा प्रतकिूल प्रभाव डाले इसअध्ययन से और प्रयोगशाला

जांच कीप्रकृतर्ि से मैं जब चाहूँ बाहर जाने के अपने अधकिार कोसुरक्षति रख रहा हूं

रोगी / रोगी के रश्तिेदार के हस्ताक्षर डॉक्टर के हस्ताक्षर या अंगूठे का नशिान

नाम: दनिांक:

मेरी उपस्थर्ति में मरीज ने पूरी तरह से डाकटर के बताए गए अध्ययन और नर्दिेशों को समझने केबाद

अपनी स्वतंत्र इच्छा से ऊपरके फर्ाम पर हस्ताक्षर कए हैं।

KEY TO MASTER CHART

S.No.-

NAME-

AGE- in years.

SEX-

OPD NO- Out patient department number

OI - OSDI score in percentage.

OI-G - OSDI grading

ST - Schirmers test value in mm.

ST-G - Schirmers test grading

TBUT- Tear film break up time in seconds.

TBUT-G – Tear film break up time grading.

VB-G – Van bijsterveld grading.

DES-G - Dry eye severity grading.

CIC-G - CIC grading.

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